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RE: New Patent Application Transmittal

Sir:

Kindly award a filing date and serial number under 35 USC 111 to the patent application based upon the enclosed specification (and any drawings). Declaration and filing fee are deferred. Please direct all correspondence to the undersigned at the address indicated below.

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TITLE: COTTON FIBER TRANSCRIPTIONAL FACTORS

[X] Specification (47 total pages including claims and abstract)

[X] 39 Sheets of Drawings

[ ] An Assignment of the invention in favor of the following organization is enclosed for recordation:

[x] Priority is hereby claimed based upon the following:

This application is a continuation-in part of of PCT/US/96/09897 FILED June 7, 1996, which is a continuation-in-part of 08/480,178 filed June 7, 1995.

[ ] Sequence Listing, Computer Readable Form and Verified Statement Under 37 CFR 1.821-1.825

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Enclosure

COTTON FIBER TRANSCRIPTIONAL FACTORS

INTRODUCTION

Technical Field

This invention relates to methods of using *in vitro* constructed DNA transcription or expression cassettes capable of directing fiber-tissue transcription of a DNA sequence of interest in plants to produce fiber cells having an altered phenotype, and to methods of providing for or modifying various characteristics of cotton fiber. The invention is exemplified by methods of using cotton fiber promoters for altering the a phenotype of cotton fiber, and cotton fibers produced by the method.

Background

In general, genetic engineering techniques have been directed to modifying the phenotype of individual prokaryotic and eukaryotic cells, especially in culture. Plant cells have proven more intransigent than other eukaryotic cells, due not only to a lack of suitable vector systems but also as a result of the different goals involved. For many applications, it is desirable to be able to control gene expression at a particular stage in the growth of a plant or in a particular plant part. For this purpose, regulatory sequences are required which afford the desired initiation of transcription in the appropriate cell types and/or at the appropriate time in the plant's development without

having serious detrimental effects on plant development and productivity. It is therefore of interest to be able to isolate sequences which can be used to provide the desired regulation of transcription in a plant cell during the growing cycle of the host plant.

One aspect of this interest is the ability to change the phenotype of particular cell types, such as differentiated epidermal cells that originate in fiber tissue, *i.e.* cotton fiber cells, so as to provide for altered or improved aspects of the mature cell type. Cotton is a plant of great commercial significance. In addition to the use of cotton fiber in the production of textiles, other uses of cotton include food preparation with cotton seed oil and animal feed derived from cotton seed husks.

Despite the importance of cotton as a crop, the breeding and genetic engineering of cotton fiber phenotypes has taken place at a relatively slow rate because of the absence of reliable promoters for use in selectively effecting changes in the phenotype of the fiber. In order to effect the desired phenotypic changes, transcription initiation regions capable of initiating transcription in fiber cells during development are desired. Thus, an important goal of cotton bioengineering research is the acquisition of a reliable promoter which would permit expression of a protein selectively in cotton fiber to affect such qualities as fiber strength, length or color.

Relevant Literature

Cotton fiber-specific promoters are discussed in PCT publications WO 94/12014 and WO 95/08914, and John and Crow, Proc. Natl. Acad. Sci. USA, 89:5769-5773, 1992. cDNA clones that are preferentially expressed in cotton fiber have been isolated. One of the clones isolated corresponds to mRNA and protein that are highest during the late primary cell wall and early secondary cell wall synthesis stages. John and Crow, *supra*.

U.S. Patent No. 5,175,095 describes tomato transcriptional factors which can be used to direct the transciption of DNA in ovary tissue. The factors are expressed immediately prior to anthesis and through flowering.

A class of fruit-specific promoters expressed at or during anthesis through fruit development, at least until the beginning of ripening, is discussed in European Application 88.906296.4, the disclosure of which is hereby incorporated by reference. cDNA clones from tomato displaying differential expression during fruit development have been isolated and characterized (Mansson *et al., Mol. Gen. Genet.* (1985) 200:356-361: Slater *et al., Plant Mol. Biol.* (1985) 5:137-147). These studies have focused primarily on mRNAs which accumulate during fruit ripening. One of the proteins encoded-by the ripening-specific cDNAs has been identified as polygalacturonase (Slater *et al., Plant Mol. Biol.* (1985) 5:137-147).

A cDNA clone which encodes tomato polygalacturonase has been sequenced (Grierson *et al., Nucleic Acids Research* (1986) 14:8395-8603). Improvements in aspects of tomato fruit storage

and handling through transcriptional manipulation of expression of the polygalacturonase gene have been reported (Sheehy *et al.*, *Proc. Natl. Acad. Sci.* USA (1988) 85:8805-8809; Smith *et al.*, *Nature* (1988) 334: 724-726).

Mature plastid mRNA for psbA (one of the components of photosystem II) reaches its highest level late in fruit development, whereas after the onset of ripening, plastid mRNAs for other components of photosystem I and II decline to nondetectable levels in chromoplasts (Piechulla *et al.*, *Plant Molec. Biol.* (1986) 7:367-376). Recently, cDNA clones representing genes apparently involved in tomato pollen (McCormick *et al.*, *Tomato Biotechnology* (1987) Alan R. Liss, Inc., NY) and pistil (Gasser *et al.*, *Plant Cell* (1989), 1:15-24) interactions have also been isolated and characterized.

Other studies have focused on genes inducibly regulated, e.g. genes encoding serine proteinase inhibitors, which are expressed in response to wounding in tomato (Graham *et al.*, *J. Biol. Chem.* (1985) 260:6555-6560: Graham *et al.*, *J. Biol. Chem.* (1985) 260:6561-6554) and on mRNAs correlated with ethylene synthesis in ripening fruit and leaves after wounding (Smith *et al.*, *Planta* (1986) 168: 94-100). Accumulation of a metallocarboxypeptidase inhibitor protein has been reported in leaves of wounded potato plants (Graham *et al.*, *Biochem & BioPhys. Res Comm.* (1981) 101: 1164-1170).

Genes which are expressed preferentially in plant seed tissues, such as in embryos or seed coats, have also been reported. See, for example, European Patent Application

87306739.1 (published as 0 255 378 on February 3, 1988) and Kridl *et al.* (Seed Science Research (1991) 1:209-219).

In animals, the *ras* superfamily is subdivided into the subfamilies *ras* which is involved in controlling cell growth and division, *rab/YPT* members which control secretory processes, and *rho* which is involved in control of cytoskeletal organization (Bourne et al., (1991) Nature 349: 117-127), and number of homologous genes have now been identified in plants (for a review, see Terryn et al., (1993) Plant Mol. Biol. 22: 143-152). None have been found for the important *ras* subfamily, all but one of the genes identified have been members of the *rab/YPT1* subfamily, and there is only one recent report of the cloning of a *rho* gene in pea (Yang and Watson(1993) Proc. Natl. Acad. Sci. USA 90: 8732-8736).

Little work has been done to characterize the functions of these genes in plants, although one recent report has shown that a small G protein from Arabidopsis can functionally complement a mutant form in yeast involved in vesicle trafficking, suggesting a similar function for the plant gene (Bednarek et al., (1994) Plant Physiol 104: 591-596).

In animals, two members of the *rho* subfamily, called Rac and Rho, have been shown to be involved in the regulation of actin organization (for a review, see Downward, (1992) Nature 359: 273-274).

Rac1 has been shown to mediate growth factor-induced membrane ruffling by influencing microfilament alignment on the plasma membrane (Ridley et al, (1992) Cell 70: 401-410), whereas

RhoA regulates the formation of actin stress fibers associated with focal adhesions (Ridley and Hall, (1992) Cell 70: 389-399).

In yeast, the CDC42 gene codes for a *rho*-type protein which also regulates actin organization involved in the establishment of cell polarity required for the localized deposition of chitin in the bud scar (Adams et al., (1990) J Cell Biol 111: 131-143.

Disruption of gene function, either by temperature shifts with a CDC42-temperature-sensitive mutant in yeast (Adams et al., 1990), or by micro-injection into fibroblasts of mutant Rac or Rho proteins exibiting a dominant negative phenotype (Ridley et al., 1992; Ridley and Hall, 1992), leads to disorganization of the actin network.

In plants, control of cytoskeletal organization is poorly understood in spite of its importance for the regulation of patterns of cell division, expansion, and subsequent deposition of secondary cell wall polymers. The cotton fiber represents an excellent system for studying cytoskeletal organization. Cotton fibers are single cells in which cell elongation and secondary wall deposition can be studied as distinct events. These fibers develop synchronously within the boll following anthesis, and each fiber cell elongates for about 3 weeks, depositing a thin primary wall (Meinert and Delmer, (1984) Plant Physiol. 59: 1088-1097; Basra and Malik, (1984) Int Rev of Cytol 89: 65-113). At the time of transition to secondary wall cellulose synthesis, the fiber cells undergo a synchronous shift in the pattern of cortical microtubule and cell wall microfibril alignments, events which may be regulated upstream by the organization of actin

(Seagull, (1990) Protoplasma 159: 44-59; and (1992) In: Proceedings of the Cotton Fiber Cellulose Conference, National Cotton Council of America, Memphis RN, pp 171-192.

Agrobacterium-mediated cotton transformation is described in Umbeck, United States Patents Nos. 5,004,863 and 5,159,135 and cotton transformation by particle bombardment is reported in WO 92/15675, published September 17, 1992. Transformation of *Brassica* has been described by Radke *et al.* (Theor. Appl. Genet. (1988) 75;685-694; Plant Cell Reports (1992) 11:499-505.

SUMMARY OF THE INVENTION

Novel DNA constructs and methods for their use are described which are capable of directing transcription of a gene of interest in cotton fiber, particularly early in fiber development and during secondary cell wall development. The novel constructs include a vector comprising a transcriptional and translational initiation region obtainable from a gene expressed in cotton fiber and methods of using constructs including the vector for altering fiber phenotype.

Two promoters are provided from genes involved in the regulation of cotton fiber development. One, Rac13, is from a protein in cotton which codes for an animal Rac protein homolog. Rac13, shows highly-enhanced expression during fiber development. This pattern of expression correlates well with the timing of

reorganization of the cytoskeleton, suggesting that the Rac13 cotton gene may, like its animal counterpart, be involved in the signal transduction pathway for cytoskeletal organization.

The other is a promoter from a cotton protein which is unrelated to published proteins, designated 4-4. 4-4 mRNA accumulates in fiber cells at day 17 post anthesis and continues to fiber maturity at days 35 post anthesis.

The methods of the present invention include transfecting a host plant cell of interest with a transcription or expression cassette comprising a cotton fiber promoter and generating a plant which is grown to produce fiber having the desired phenotype. Constructs and methods of the subject invention thus find use in modulation of endogenous fiber products, as well as production of exogenous products and in modifying the phenotype of fiber and fiber products. The constructs also find use as molecular probes. In particular, constructs and methods for use in gene expression in cotton embryo tissues are considered herein. By these methods, novel cotton plants and cotton plant parts, such as modified cotton fibers, may be obtained.

Also provided in the instant application are constructs and methods of use relating to modification of color phenotype in fiber tissues. Such constructs contain sequences for expression of genes involved in the production of colored compounds, such as anthocyanins, melanin or indigo, and also may contain sequences which provide for targeting of the gene products to particular locations in the plant cell, such as plastid organelles, or vacuoles. Plastid targeting is of particular interest for

expression of genes involved in aromatic amino acid biosynthesis pathways, while vacuolar targeting is of particular interest where the precursors required in synthesis of the pigment are present in vacuoles. Production of melanin, for example, may be enhanced by vacuolar targeting in plant tissues which accumulate tyrosine in vacuoles. Transcriptional initiation regions for expression of color-related genes will be selected on the basis of the tissue for which color modification is desired.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows the DNA sequence encoding the structural protein from cDNA 4-4.

Figure 2 shows the sequence to the promoter construct pCGN5606 made using genomic DNA from 4-4-6 genomic clone.

Figure 3 shows the sequence to the 4-4 promoter construct pCGN5610.

Figure 4 shows the cDNA sequence encoding the Rac13 gene expressed in cotton fiber.

Figure 5 shows the sequence the promoter region from the *rac*13 gene.

Figure 6 shows a restriction map for pCGN4735.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the subject invention, novel constructs and methods for their use are described which may be used as molecular probes or inserted into a plant host to provide for transcription of a nucleotide sequence of interest in fiber cells

as compared with other plant cells, generally preferentially in fiber cells to produce cells and plant parts having an altered phenotype. Of particular interest is the period of at least one to three days prior to anthesis through flower senescence. One promoter was derived from the characterization of two distinct *rac* cDNA clones isolated from a cotton fiber cDNA library which code for homologs of animal Rac proteins. Using gene-specific probes, it was determined that amphidiploid cotton contains two genes that code for each of the two *rac* proteins, designated Rac13 and Rac9 respectively. The gene for Rac13 shows highly-enhanced expression in developing cotton fibers, with maximal expression occuring at the time of transition between primary and secondary wall synthesis. This is also the time at which reorganization of the cytoskeleton occurs, and thus the pattern of expression of Rac13 is consistent with its possible role, analogous to animal Rac, in the signal transduction pathway for cytoskeletal organization.

The constructs may include several forms, depending upon the intended use of the construct. Thus, the constructs include vectors, transcriptional cassettes, expression cassettes and plasmids. The transcriptional and translational initiation region (also sometimes referred to as a "promoter,"), preferably comprises a transcriptional initiation regulatory region and a translational initiation regulatory region of untranslated 5' sequences, "ribosome binding sites," responsible for binding mRNA to ribosomes and translational initiation. It is preferred that all of the transcriptional and translational functional elements

of the initiation control region are derived from or obtainable from the same gene. In some embodiments, the promoter will be modified by the addition of sequences, such as enhancers, or deletions of nonessential and/or undesired sequences. By "obtainable" is intended a promoter having a DNA sequence sufficiently similar to that of a native promoter to provide for the desired specificity of transcription of a DNA sequence of interest. It includes natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences.

The vectors will comprise a nucleotide sequence comprising the transcriptional initiation regulatory regions of this invention associated. A transcriptional cassette for transcription of a nucleotide sequence of interest in cotton fiber will include in the direction of transcription, the cotton fiber transcriptional initiation region, a DNA sequence of interest, and a transcriptional termination region functional in the plant cell. When the cassette provides for the transcription and translation of a DNA sequence of interest it is considered an expression cassette. One or more introns may be also be present.

Other sequences may also be present, including those encoding transit peptides and secretory leader sequences as desired. The regulatory regions are capable of directing transcription in fiber cells from anthesis through flowering but direct little or no expression after the initial changes which occur at the time surrounding pollination and/or fertilization;

transcription from these regulatory regions is not detectable at about three weeks after anthesis. Further, fiber-tissue transcription initiation regions of this invention are typically not readily detectable in other plant tissues. Transcription initiation regions from cotton fiber that are not fiber specific may find special application. Especially preferred are transcription initiation regions which are not found at stages of fiber development other than pre-anthesis through flowering. Transcription initiation regions capable of initiating transcription in other plant tissues and/or at other stages of fiber development, in addition to the foregoing, are acceptable insofar as such regions provide a significant expression level in cotton fiber at the defined periods of interest and do not negatively interfere with the plant as a whole, and, in particular, do not interfere with the development of fiber and/or fiber-related parts. Also of interest are cotton fiber promoters and/or promoter elements which are capable of directing transcription in specific cotton fibers such as outer pericarp tissue, inner core tissues, integuments, and the like.

The term "fiber" as used herein refers to the mature organ formed as the result of the development of the fiber wall of a flower and any other closely associated parts. See Weirer, T.E., 1, ed., *Botany A Introduction to Plant Biology* (6th ed.) (John Wiley & Sons, 1982); Tootill & Backmore, The Facts on File Dictionary of Botany (Market Home Books Ltd., 1984). By "modified fiber" is meant fiber having a detectably different phenotype from a nontransformed plant of the same species, for

example, one not having the transcriptional cassette in question in its genome. The term "anthesis" refers herein to the period associated with flower opening and flowering. The term "flower senescence" refers herein to the period associated with flower death, including the loss of the (flower) petals, etc. Abercrombie, *M., et al., A Dictionary of Biology* (6th ed) (Penguin Books, 1973). Unopened flowers, or buds, are considered "pre-anthesis." Anthesis begins with the opening of the flower petals, which represents asexually receptive portion of the reproductive cycle of the plant. Typically, flowering lasts approximately one week in the tested UCB82 tomato variety. In a plant like cotton, flowering lasts approximately two weeks and the fiber develops from the seed coat tissue. It is preferred that the transcriptional initiation regions of this invention do not initiate transcription for a significant time or to a significant degree prior to plant flower budding. Ideally, the level of transcription will be high for at least approximately one to three days and encompass the onset of anthesis ("pre-anthesis").

Cotton fiber is a differentiated single epidermal cell of the outer integument of the ovule. It has four distinct growth phases; initiation, elongation (primary cell wall synthesis), secondary cell wall synthesis, and maturation. Initiation of fiber development appears to be triggered by hormones. The primary cell wall is laid down during the elongation phase, lasting up to 25 days postanthesis (DPA). Synthesis of the secondary wall commences prior to the cessation of the elongation

phase and continues to approximately 40 DPA, forming a wall of almost pure cellulose. In addition to cotton fiber promoters, transcriptional initiation regions from genes expressed preferentially in seed tissues, and in particular seed coat tissues, are also of interest for applications where modification of cotton fiber cells is considered.

Downstream from, and under the regulatory control of, the cotton fiber transcriptional/translational initiation control region is a nucleotide sequence of interest which provides for modification of the phenotype of fiber. The nucleotide sequence may be any open reading frame encoding a polypeptide of interest, for example, an enzyme, or a sequence complementary to a genomic sequence, where the genomic sequence may be an open reading frame, an intron, a noncoding leader sequence, or any other sequence where the complementary sequence inhibits transcription, messenger RNA processing, for example, splicing, or translation. The nucleotide sequences of this invention may be synthetic, naturally derived, or combinations thereof. Depending upon the nature of the DNA sequence of interest, it may be desirable to synthesize the sequence with plant preferred codons. The plant preferred codons may be determined from the codons of highest frequency in the proteins expressed in the largest amount in the particular plant species of interest. Phenotypic modification can be achieved by modulating production either of an endogenous transcription or translation product, for example as to the amount, relative distribution, or the like, or an exogenous transcription or translation product, for example to provide for

a novel function or products in a transgenic host cell or tissue. Of particular interest are DNA sequences encoding expression products associated with the development of plant fiber, including genes involved in metabolism of cytokinins, auxins, ethylene, abscissic acid, and the like. Methods and compositions for modulating cytokinin expression are described in United States Patent No. 5,177,307, which disclosure is hereby incorporated by reference. Alternatively, various genes, from sources including other eukaryotic or prokaryotic cells, including bacteria, such as those from *Agrobacterium tumefaciens* T-DNA auxin and cytokinin biosynthetic gene products, for example, and mammals, for example interferons, may be used.

Other phenotypic modifications include modification of the color of plant parts developing from fiber integuments and/or core tissue, for example seed coat hairs, such as cotton fibers. Of interest are genes involved in production of melanin and genes involved in the production of indigo. Melanins are dark brown pigments found in animals, plants and microorganisms, any of which may serve as a source for sequences for insertion into the constructs of the present invention. Specific examples include the tyrosinase gene which can be cloned from *Streptomyces antibioticus*. The ORF438 encoded protein in S. *antibioticus* also is necessary for melanin production, and may provide a copper donor function. In addition, a tyrosinase gene can be isolated from any organism which makes melanin. The gene can be isolated from human hair, melanocytes or melanomas, cuttle fish and red roosters, among others. See, for example, EP Application No.

89118346.9 which discloses a process for producing melanins, their precursors and derivatives in microorganisms. Also, See, Bernan *et al.* Gene (1985) 37:101-110; and della-Cioppa *et al.* Bio/Technology (1990) 8:634-638.

Indigo may be obtained by use of genes encoding a mono-oxygenase such as xylene oxygenase which oxidizes toluene and xylene to (methyl) benzyl alcohol and also transforms indole to indigo. Cloning of the xylene oxygenase gene and the nucleotide and amino acid sequences are described in unexamined Japanese Patent Application Kokai:2-119777, published May 7, 1990. A dioxygenase such as naphthalene dioxygenase which also converts indole to indigo finds use; the naphthalene dioxygenase gene nahA is described in Science (1983) 222: 167. For cloning, nucleotide sequence in characterization of genes encoding naphthalene dioxygenase of Pseudomonas putida. See, Kurkela *et al.* Gene (1988) 73:355-362. A tryptophanase gene sequence can be used in conjunction with an oxygenase to increase the amount of indole available for conversion to indigo. Sources of tryptophanase gene sequences include *E. coli* (see, for example, Deeley *et al.* (1982) *J. Bacteriol.* 151 :942-951).

As demonstrated in the copending application to McBride *et al.*, entitled "Use of Ovary Tissue Transcriptional Factors", serial no. 08/480,178, filed on June 7, 1995, the teachings of which are incorporated herein by reference, expression of ORF438 and tyrosinase genes from *Streptomyces* in transgenic tobacco plants using a 4-4 and rac promoter, and targeting the gene products to plastids by the action of transit peptides resulted

in phenotypic modification of ovary derived and meristem derived tissues, including modification of color in meristematic regions and basal flower buds. A similar set of experiments in which no plastid targeting sequences were used in conjunction with the ORF438 and tyrosinase genes, no alteration of phenotype was observed. Presumably, the plants were not able to produce melanin due to deficiency of the required substrates in the plant cell cytosol.

Plastid targeting sequences (transit peptides) are available from a number of plant nuclear-encoded plastid proteins, such as the small subunit (SSU) of ribulose bisphosphate carboxylase, plant fatty acid biosynthesis related genes including acyl carrier protein (ACP), stearoyl-ACP desaturase, ß-ketoacyl-ACP synthase and acyl-ACP thioesterase, or LHCPII genes. The encoding sequence for a transit peptide which provides for transport to plastids may include all or a portion of the encoding sequence for a particular transit peptide, and may also contain portions of the mature protein encoding sequence associated with a particular transit peptide. There are numerous examples in the art of transit peptides which may be used to deliver a target protein into a plastid organelle. The particular transit peptide encoding sequence used in the instant invention is not critical, as long as delivery to the plastid is obtained.

As an alternative to using transit peptides to target pigment synthesis proteins to plastid organelles, the desired constructs may be used to transform the plastid genome directly.

In this instance, promoters capable of providing for transcription of genes in plant plastids are desired. Of particular interest is the use of a T7 promoter to provide for high levels of transcription. Since plastids do not contain an appropriate polymerase for transcription from the T7 promoter, T7 polymerase may be expressed from a nuclear construct and targeted to plastids using transit peptides as described above. (See McBride *et al.* (1994) *Proc. Nat. Acad. Sci. 91*:7301-7305; see also copending US patent application entitled "Controlled Expression of Transgenic Constructs in Plant Plastids", serial no. 08/472,719 filed June 6, 1995, and copending US patent application SN 08/167,638, filed December 14, 1993 and PCT/US94/14574 filed December 12, 1994.) Tissue specific or developmentally regulated promoters may be useful for expression of the T7 polymerase in order to limit expression to the appropriate tissue or stage of development. For example, for flower color modification, the T7 polymerase may be expressed from a petal specific promoter to limit effects to the desired tissue.

Targeting of melanin synthesis genes to vacuoles is also of interest in plant tissues which accumulate the tyrosine substrate involved in melanin synthesis in vacuoles. The protein signal for targeting to vacuoles may be provided from a plant gene which is normally transported across the rough endoplasmic reticulum, such as the 32 amino acid N-terminal region of the metallocarboxypeptidase inhibitor gene from tomato (Martineau *et al.* (1991) *Mol. Gen. Genet. 228* :281-286). In addition to the

signal sequence, vacuolar targeting constructs also encode a vacuolar localization signal (VLS) positioned at the carboxy terminus of the encoded protein. Appropriate signal sequences and VLS regions may be obtained from various other plant genes and may be similarly used in the constructs of this invention. Numerous vacuolar targetting peptides are known to the art, as are reviewed in Chrispeels *et al.*, Cell (1992) 68:613-616.

Thus, it is recognized that constructs of the instant invention which provide sequences encoding genes involved in color production and sequences which provide for targeting of the gene products to appropriate cellular locations have broad application to modification of color in various plant tissues. Plant transcriptional initiation regions for use with these color modification constructs will depend upon the particular plant tissue to be modified. For cotton fiber modification the 4-4 and *rac*13 cotton fiber promoters may find use.

Also of interest are genes involved in production of colored pigments in plant tissues. The Maize A1 gene which encodes a dihydroflavonol reductase, an enzyme of the anthocyanin pigmentation pathway is one such gene. In cells that express the A1 gene, dihydrokempferol is converted to 2-8 alkylleucopelargonidin, which may be further metabolized to pelargonidin pigment by endogenous plant enzymes. Other anthocyanin or flavonoid type pigments may also be of interest for modification of cotton cell fibers, and have been suggested for use in plant flowers (for a review of plant flower color, see van Tunen *et al.*, Plant Biotechnology Series, Volume 2 (1990)

Developmental Regulation of Plant Gene Expression, D. Grierson ed.). Anthocyanin is produced by a progression of steps from cellular phenylalanine pools. The R anc C1 genes are maize regulatory proteins which are active by positively affecting upstream steps in the anthocyanin biosynthesis from these pools. The R gene is described in Perot and Cone (1989) Nucl. Acids Res., 17:8003, and the C1 gene is described in Paz-Ares *et al* (1987) EMBO, 6:3553-3558. Lloyd *et al.* (1992) Science, 258:1773-1775 discussed both genes.

Although cotton fibers in commercially grown varieties are primarily white in color, other naturally occurring cotton varieties have brown or reddish-brown fibers. Also a cotton line containing green colored fibers has been identified. The existence of these colored cotton lines suggests that the precursors required for the anthocyanin pigment pathways are present in cotton fibers cells, thus allowing further color phenotype modifications. Thus, the maize R and C1 genes could be used in enhancing the levels of of anthocyanin produced in fiber cells. As the R and C1 proteins are proteins with a positive control at the regulatory level on anthocyanin pigment precursor biosynthesis, these proteins are expressed in the nucleus, and not targetted to plastids or vacuoles.

For some applications, it is of interest to modify other aspects of structures developing from the fiber integument and related structures. For example, it is of interest to modify various aspects of cotton fibers, such as strength or texture of a fiber. Thus, the appropriate gene may be inserted in the

constructs of the invention, including genes for PHB biosynthesis (see, Peoples *et al. J. Biol. Chem.* (1989) 264: 15298-15303 and *Ibid.* 15293-15397; Saxena, *Plant Molecular Biology* (1990) 15:673-683, which discloses cloning and sequencing of the cellulose synthase catalytic subunit gene; and Bowen *et al. PNAS* (1992) 89:519-523 which discloses chitin synthase genes of *Saccharomyces cerevisiae* and *Candida albicans.* Various constructs and methods are disclosed for the use of hormones to effect changes to fiber quality in copending US patent application entitled "Cotton Modification Using Ovary-Tissue Transcriptional factors", serial no. 08/397,652 filed February 2, 1995, the teachings of which are incorporated herein by reference.

Transcriptional cassettes may be used when the transcription of an anti-sense sequence is desired. When the expression of a polypeptide is desired, expression cassettes providing for transcription and translation of the DNA sequence of interest will be used. Various changes are of interest; these changes may include modulation (increase or decrease) of formation of particular saccharides, hormones, enzymes, or other biological parameters. These also include modifying the composition of the final fiber or fiber, that is changing the ratio and/or amounts of water, solids, fiber or sugars. Other phenotypic properties of interest for modification include response to stress, organisms, herbicides, brushing, growth regulators, and the like. These results can be achieved by providing for reduction of expression of one or more endogenous products, particularly an enzyme or cofactor, either by producing a transcription product which is

complementary (anti-sense) to the transcription product of a native gene, so as to inhibit the maturation and/or expression of the transcription product, or by providing for expression of a gene, either endogenous or exogenous, to be associated with the development of a plant fiber.

The termination region which is employed in the expression cassette will be primarily one of convenience, since the termination regions appear to be relatively interchangeable. The termination region may be native with the transcriptional initiation region, may be native with the DNA sequence of interest, may be derived from another source. The termination region may be naturally occurring, or wholly or partially synthetic. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens,* such as the octopine synthase and nopaline synthase termination regions. In some embodiments, it may be desired to use the 3′ termination region native to the cotton fiber transcription initiation region used in a particular construct.

As described herein, in some instances additional nucleotide sequences will be present in the constructs to provide for targeting of a particular gene product to specific cellular locations. For example, where coding sequences for synthesis of aromatic colored pigments are used in a construct, particularly coding sequences for enzymes which have as their substrates aromatic compounds such tyrosine and indole, it is preferable to include sequences which provide for delivery of the enzyme into plastids, such as an SSU transit peptide sequence. Also, for

synthesis of pigments derived from tyrosine, such as melanin, targeting to the vacuole may provide for enhanced color modifications.

For melanin production, the tyrosinase and ORF438 genes from *Streptomyces antibioticus (Berman et al.* (1985) 37:101-110) are provided in cotton fiber cells for expression from a 4-4 and Rac13 promoter. In *Streptomyces,* the ORF438 and tyrosinase proteins are expressed from the same promoter region. For expression from constructs in a transgenic plant genome, the coding regions may be provided under the regulatory control of separate promoter regions. The promoter regions may be the same or different for the two genes. Alternatively, coordinate expression of the two genes from a single plant promoter may be desired. Constructs for expression of the tyrosinase and ORF438 gene products from 4-4 and rac promoter regions are described in detail in the following examples. Additional promoters may also be desired, for example plant viral promoters, such as CaMV 35S, can be used for constitutive expression of one of the desired gene products, with the other gene product being expressed in cotton fiber tissues from the 4-4 and rac promoter. In addition, the use of other plant promoters for expression of genes in cotton fibers is also considered, such as the *Brassica* seed promoters and the E6 gene promoter discussed above. Similarly, other constitutive promoters may also be useful in certain applications, for example the mas, Mac or DoubleMac, promoters described in United States Patent No. 5,106,739 and by Comai *et al., Plant Mol. Biol.* (1990) *15:373-381).* When plants comprising

multiple gene constructs are desired, for example plants expressing the melanin genes, ORF438 and tyrosinase, the plants may be obtained by co-transformation with both constructs, or by transformation with individual constructs followed by plant breeding methods to obtain plants expressing both of the desired genes.

Color constructs which may find use in the methods of the instant application are described in copending US patent application to McBride *et al.*, *supra*. Constructs for melanin and indigo expression are described therein, as well as results showing melanin expression in plant cells.

A variety of techniques are available and known to those skilled in the art for introduction of constructs into a plant cell host. These techniques include transfection with DNA employing *A. tumefaciens* or *A. rhizogenes* as the transfecting agent, protoplast fusion, injection, electroporation, particle acceleration, etc. For transformation with *Agrobacterium,* plasmids can be prepared in *E. coli* which contain DNA homologous with the Ti-plasmid, particularly T-DNA. The plasmid may or may not be capable of replication in *Agrobacterium,* that is, it may or may not have a broad spectrum prokaryotic replication system such as does, for example, pRK290, depending in part upon whether the transcription cassette is to be integrated into the Ti-plasmid or to be retained on an independent plasmid. The *Agrobacterium* host will contain a plasmid having the *vir* genes necessary for transfer of the T-DNA to the plant cell and may or may not have the complete TDNA. At least the right border and

frequently both the right and left borders of the T-DNA of the Ti- or Ri-plasmids will be joined as flanking regions to the transcription construct. The use of T-DNA for transformation of plant cells has received extensive study and is amply described in EPA Serial No. 120,516, Hoekema, In: The Binary Plant Vector System Offset-drukkerij Kanters B.V., Alblasserdam, 1985, Chapter V, Knauf, *et al.*, Genetic Analysis of Host Range Expression by *Agrobacterium,* In: Molecular Genetics of the Bacteria-Plant Interaction, Puhler, A. ed., Springer-Verlag, NY, 1983, p. 245, and An, *et al., EMBO J.* (1985) 4:277-284.

For infection, particle acceleration and electroporation, a disarmed Ti-plasmid lacking particularly the tumor genes found in the T-DNA region) may be introduced into the plant cell. By means of a helper plasmid, the construct may be transferred to the *A. tumefaciens* and the resulting transfected organism used for transfecting a plant cell; explants may be cultivated with transformed *A. tumefaciens* or *A. rhizogenes* to allow for transfer of the transcription cassette to the plant cells. Alternatively, to enhance integration into the plant genome, terminal repeats of transposons may be used as borders in conjunction with a transposase. In this situation, expression of the transposase should be inducible, so that once the transcription construct is integrated into the genome, it should be relatively stably integrated. Transgenic plant cells are then placed in an appropriate selective medium for selection of transgenic cells which are then grown to callus, shoots grown and plantlets generated from the shoot by growing in rooting medium.

To confirm the presence of the transgenes in transgenic cells and plants, a Southern blot analysis can be performed using methods known to those skilled in the art. Expression products of the transgenes can be detected in any of a variety of ways, depending upon the nature of the product, and include immune assay, enzyme assay or visual inspection, for example to detect pigment formation in the appropriate plant part or cells. Once transgenic plants have been obtained, they may be grown to produce fiber having the desired phenotype. The fiber or fiber parts, such as cotton fibers may be harvested, and/or the seed collected. The seed may serve as a source for growing additional plants having the desired characteristics. The terms transgenic plants and transgenic cells include plants and cells derived from either transgenic plants or transgenic cells.

The various sequences provided herein may be used as molecular probes for the isolation of other sequences which may be useful in the present invention, for example, to obtain related transcriptional initiation regions from the same or different plant sources. Related transcriptional initiation regions obtainable from the sequences provided in this invention will show at least about 60% homology, and more preferred regions will demonstrate an even greater percentage of homology with the probes. Of particular importance is the ability to obtain related transcription initiation control regions having the timing and tissue parameters described herein. For example, using the probe 4-4 and rac, at least 7 additional clones, have been identified, but not further characterized. Thus, by employing the techniques

described in this application, and other techniques known in the art (such as Maniatis, *et al., Molecular Cloning,- A Laboratory Manual* (Cold Spring Harbor, New York) 1982), other transcription initiation regions capable of directing cotton fiber transcription as described in this invention may be determined. The constructs can also be used in conjunction with plant regeneration systems to obtain plant cells and plants; the constructs may also be used to modify the phenotype of a fiber and fibers produced thereby.

Various varieties and lines of cotton may find use in the described methods. Cultivated cotton species include *Gossypium hirsutum* and *G. babadense* (extra-long stable, or Pima cotton), which evolved in the New World, and the Old World crops *G. herbaceum* and *G. arboreum.*

The following examples are offered by way of illustration and not by limitation.

EXPERIMENTAL

Example 1

cDNA libraries

Tissue preparation for cDNA synthesis

Leaf and root tissue were isolated from 8 inch tall greenhouse grown seedlings and immediately frozen in liquid nitrogen. Flowers were collected at the rapidly expanding 3 day preanthesis stage and also frozen. Seed was collected from 21 day postanthesis locules which had been removed from the boll and

frozen entire in liquid nitrogen. Once frozen, the fiber was removed from the seed and the denuded seed used for RNA isolation. All fibers were removed from the seed under liquid nitrogen and the fiber was ground to a powder prior to RNA isolation. Fibers were from bolls which had been tagged at anthesis.

DNA and RNA Manipulations

The lZapII cDNA library used for screening was prepared from cDNA derived from poly-A^+ mRNA isolated from fibers of *Gossypium hirsutum* cultivar Acala SJ-2. The fibers were isolated from bolls harvested at approximately 21 dpa using field-grown plants in Israel.

Total RNA was isolated from 21 dpa seeds (*G. hirsutum* cv Coker 130 from which the fiber had been removed) using the method of Hughes and Galau ((1988) Plant Mol Biol Reporter, 6:253-257.) All other RNAs were prepared according to Hall *et al.* ((1978), Proc Natl Acad Sci USA 75: 3196-3200), with the following modifications. After the second 2M LiCl wash, the pellet was dissolved in 1/10 original volume of 10 mM Tris pH7.5 and brought to 35mM potassium acetate pH6.5 and 1/2 volume EtOH was added slowly. The mixture was placed on ice for 15 minutes and then centrifuged at 20,000 x *g* for 15 minutes at $4^{O}C$. The potassium acetate concentration was brought to 0.2M, 2 1/2 volumes EtOH added and the RNA placed at $-20^{O}C$ for several hours. The precipitate was centrifuged at 12,000 x *g* for 30 minutes at $4^{O}C$ and the pellet was resuspended in diethylpyrocarbonate-treated water. Poly-A^+ RNA was prepared from total mRNA utilizing an

oligo(dT)-cellulose kit (Becton Dickenson) and following the manufacturer's protocol.

Cotton genomic DNA was prepared as follows. Four grams of young cotton leaf tissue (cv Coker 130) was ground to a powder in N_2 and placed in an Oak Ridge tube with 0.4g polyvinylpyrolidone and 20mls extraction buffer (200mM Ches/NaOH ph9.1, 200mM NaCl, 100mMEDTA/NaOH pH9.0, 2% SDS, 0.5% Na deoxycholate, 2% Nonidet NP-40, 20mM B-mercaptoethanol) was added to sample, gently mixed and incubated at $65^{O}C$ in a shaking water bath for 10 minutes. 7.0 mls of 5M potassium acetate pH6.5 was added and carefully mixed. Incubation was carried out on ice for 30 minutes with gentle mixing every 5 minutes. The sample was centrifuged for 20 minutes at 21,000 x g and the supernatant was filtered through Miracloth into another tube and centrifuged as before. The supernatant was again filtered through Miracloth into 15 mls of room temperature isopropanol in an Oak Ridge tube. After gentle mixing, the sample was incubated at room temperature for 10-60 minutes until the DNA precipitated. The DNA was spooled and allowed to air dry before being resuspended in 4 mls of TE on ice for 1 hour. CsCl was added to 0.97g/ml final concentration and 300 ul 10mg/ml ethidium bromide was also added before filling VTi80 quick seal tubes. The sample was centrifuged overnight at 225,000 x g overnight. The DNA was extracted with water saturated butanol and enough water was added to bring the volume to 4 mls before adding 2 volumes EtOH. The DNA was spooled, air dried and resuspended in 200 ul sterile water.

<u>Northern and Southern Analysis</u>

For Northerns, 10ug of total RNA was isolated from various tissues, separated by electrophoresis in 1.2% agarose-formaldehyde gels and transfered onto Nytran Plus membranes (Schleicher and Schuell). Hybridization conditions consisted of a solution containing 50% formamide(v/v), 5xSSC, 0.1% SDS, 5mM EDTA, 10X Denhardts solution, 25mM sodium phosphate pH6.5 and 250 ug/ml carrier DNA. Washes were performed in 2xSSC, 0.1% SDS at $42^{O}C$ 3 times for 30 minutes each time.

Cotton genomic DNA (12ug) was digested with various restriction endonucleases, electrophoresed in 0.9% agarose gels and blotted onto Nytran Plus membranes. Hybridization and filter washing conditions for both the 3′ specific and full-length cDNA insert probes were as described for Northern analysis.

Probes derived from 3′-untranslated regions were synthesized via oligonucleotide primers from the Rac13 cDNA, corresponding to bases 600-619 and 843-864 (Figure 4). Each set of primers was used in a polymerase chain reaction to synthesize copies of 3′-specific DNA sequences. These sequences were used as templates in the generation of single-stranded, ^{32}P-labeled probes off the antisense strand in a polymerase chain reaction. The full-length cDNA inserts for Rac13 were used as templates for double stranded, random primed probes using the Prime-It kit (Stratagene).

Example 2

Isolation of cDNA Clones from Cotton

cDNA to the 4-4 clone was isolated from the cotton fiber library described above, and shown to express in fiber but not other tissues. This sequence was not related to any known protein. Only 400 kb of encoding sequence was present in this clone, so the library was rescreened using the cDNA to obtain full-length clones. The full-length encoding sequence is provided in Figure 1.

Another clone was sequenced which showed high homology to animal Rac proteins. This clone, designated Rac13, was not quite full-length, and the library was re-screened using this initial Rac13 DNA segment as probe. Of approximately 130,000 primary plaques screened, 56 screened positive; of these, 14 clones were isolated and sequenced. Of these 14 clones, 12 showed identical sequence homology to the original Rac13 clone and one of these cDNA clones encoded a full length Rac13. One other partial-length cDNA clone, designated Rac9, was clearly related, but distinct in DNA and amino acid sequence from Rac13. Re-screening of 150,000 plaques resulted in the isolation of 36 positive clones of which only two clones corresponded to the Rac9 sequence (both full-length clones), the remainder being Rac13. These results suggest that cotton contains genes for at least two distinct Rac proteins. Based upon the frequency of clone isolation, Rac13 is relatively highly-expressed and Rac9 less so in cotton fibers at 21 days post-anthesis (dpa), the age at which polyA$^+$ mRNA was isolated for library construction.

Figure 4 shows the DNA and deduced amino acid sequences for Rac13 full length. Comparisons of the deduced amino acid

sequence of Rac13 with other small G-proteins showed that the cotton Rac proteins are very closely related to the Rho1 protein sequence deduced from a cDNA clone isolated recently from pea (Yang and Watson, *supra*). After the pea Rho1, mammalian Rac proteins show the highest homology with the cotton Rac proteins. Other proteins of the *rho* subfamily, such as the yeast CDC42 and human RhoA, are also clearly related to the cotton Rac genes. By contrast, the other small G-proteins of the *Rab/YPT* subfamily isolated from plants such as the example shown of the tobacco RAB5 protein, as well as the human Ras proteins, are least homologous to the cotton Rac proteins of all the small G-proteins compared. The cotton and pea proteins, as well as the mammalian Racs, all have pI's above 9, whereas those of other *rho* and *ras* proteins are in the range of 5.0-6.5.

Example 3

<u>Expression of Cotton Fiber Genes in Developing Fibers</u>

Expression of the Rac13 and 4-4 genes was assessed using mRNA prepared from various cotton tissues and from fibers at different stages of development. Blots were hybridized with probes derived from 3'-untranslated regions of either the Rac13 or 4-4 genes. The gene for Rac13 exhibits highly-enhanced expression in fibers; virtually no detectable mRNA is present in leaves, roots, or flower parts, even under conditions of extended development time. Rac13 expression is detected in seeds at an age that corresponds to the highest expression levels observed in fiber tissue derived from seeds of this same age. The pattern of

Rac13 expression in fibers is very dependent upon the developmental stage. Expression is very low during the stage of primary wall synthesis (0-14 dpa, see Meinert and Delmer, 1977), reaches a maximum during the transition to secondary wall synthesis (about 15-18 dpa), and declining during the stage of maximal secondary wall cellulose synthesis (about 24-28 dpa).

4-4 mRNA is begins to accumulate in fiber cells only at day 17 post anthesis and continues through fiber maturity at day 35 post anthesis. Levels peak at day 21 and remain high throughout fiber maturation to 35 days post anthesis. 4-4 mRNA is not detected in other cotton tissues, and is not detected in fiber tissue before onset at 17 days post anthesis.

Example 4

Genomic DNA

cDNA for both the 4-4 and Rac13 was used to probe for genomic clones. For both, full length genomic DNA was obtained from a library made using the lambda dash 2 vector from Stratagene™, which was used to construct a genomic DNA library from cotton variety Coker 130 (*Gossypium hirsutum* cv. coker 130), using DNA obtained from germinating seedlings.

Example 5

Preparation of 4-4 Promoter Constructs

pCGN5606

The pCGN5606 promoter construct comprises the 4-4 cotton fiber expression cassette in a first version, version I (Figure 2). The sequences from nt1 to 65 and nt 5,494 to 5,547 correspond to fragments of the pBluescriptII polylinker where this cassette is cloned. Unique restriction enzyme sites present in these regions flanking the cassette allow the cloning of the fiber expression cassette into binary vectors including the pCGN 5138 and 1547 series.

The sequences from nt57 to 5,494 are contained in a lambda phage clone of a cotton Coker 130 genomic library. This clone is described in my notebook as lambda genomic clone 4-4(6). P462003 page 18 and following.

The region from nt 65 to nt 4,163 corresponds to the 5′ flanking region of the 4-4(6) gene. At nt 4,163 there is a NcoI restriction site sequence that corresponds to the first codon of the 4-4 (6)ORF.

The region from nucleotide 4,163 to 4,502 corresponds to part of the 4-4 (6)ORF. The sequence from nt 4,502 to 4,555 is a synthetic polylinker oligonucleotide that contains unique target sites for the restriction enzymes EcoRI, SmaI, SalI, NheI and BglII. This fragment from nt4,163 to 4,555 is a stuffer fragment and is left in place to facilitate the monitoring of cloning manipulations.

The genes to be expressed in cotton fiber cells using this cassette can be cloned between the NcoI restriction site and any of the polylinker sites. This operation will replace the stuffer fragment with the gene of interest. The region from nt 4,555to 5,494 corresponds to the 940 nucleotides downstream of the stop codon and constitute the 3′ flanking region of the 4-4 (6) gene. There is a unique AscI restriction enzyme site at nt 5483.

<u>pCGN5610</u>

The pCGN5610 construct is a second version of a 4-4 cotton fiber expression cassette, version II, which is a modified version of pCGN5606. The two versions of the 4-4 cotton fiber expression cassette are designed to allow the cloning of tandem arrays of two fiber cassettes in one binary plasmid. The differences with respect to pCGN5606 are very minor and described below.

The XbaI restriction site in the region of nt 1 to 65 has been deleted by standard cloning manipulations.

The polylinker region is in the reverse orientation of pCGN5606. There is a unique XbaI restriction enzyme site at nt5484. The sequences from nt1 to 57 and nt 5,494 to 5,518 of pCGN5610 correspond to fragments of the pBluescriptII polylinker where this cassette is cloned. Unique restriction enzyme sites present in these regions allow the cloning of the fiber expression cassette into binary vectors of the pCGN 5138 and 1547 series.

The sequences from nt57 to 5,494 are contained a lambda phage clone of a Coker 130 genomic library. This clone is described in my notebook as lambda genomic clone 4-4(6). The

region from nt 57 to nt 4,155 corresponds to the 5′ flanking region. At nt 4,155 there is a NcoI restriction site sequence that corresponds to the first codon of the 4-4 ORF.
The region from nucleotide 4,156 to 4,500 corresponds to part of the 4-4 ORF. This fragment from nt4,156 to 4,550 is a stuffer fragment and is left in place to facilitate the monitoring of cloning manipulations. The sequence from nt 4,500 to 4,550 is a synthetic polylinker oligonucleotide containing unique target sites for the restriction enzymes BglII, NheI, SalI, SmaI and EcoRI.

The genes to be expressed in cotton fiber cells using this cassette can be cloned between the NcoI restriction site and any of the polylinker sites . This operation will replace the stuffer fragment with the gene of interest. The region from nt 4,550 to 5,494 corresponds to the 940 nucleotides downstream of the stop codon and constitute the 3′ flanking region of the 4-4 (6) gene.

Example 6

Preparation of Rac13 Promoter Constructs

Genomic clone

From a genomic clone designated 15-1, mapping was done with restriction endonucleases. The largest fragment with the Rac13 coding region was identified. Theis was a Pst fragment, and when subcloned in the Bluescript™ KS+ vector (BSKS+; Stratagene) was named pCGN4722. The insert had a length of 9.2 kb.

The region of the Pst fragment with the Rac13 coding sequence was identified. DNA sequence was determined for approximately 1.7 kb 5' of the start codon and approximately 1.2 kb 3' of the stop codon. The entire Rac coding region (exons and introns) was conveniently flanked by Nde1 sites.

pCGN4722 was digested with Xba1, and a 2.7 kb fragment was removed. Religation gave pCGN4730, which was then digested with Nde1, dropping out a 1.7 kb fragment containing the entire Rac coding region. Religation yielded pCGN4731.

A polylinker region was created using overlapping synthetic oligonucleotides which were PCR'ed using primers homologous to the 5' and 3' ends of the resynthesized section. The resulting product was digested with EcooR1 and Hind III and ligated into BSKS+ at eht EcoR1 and Hind III sites. The resulting plasmid was designated pCGN4733.

pCGN4731 and pCGN4633 were digested with Nde! and the Nde1 fragment containing the synthesized polylinker region from pCGN4733 was dropped in the Nde1 site of 4731, giveing pCGN4734. This last plasmid was digested with Sal and Xba, ans so was

pCGN5133. pCGN5133 was the 9.2 kb pst fragment in BSKS+ where the polylinker sites flanking the insert were altered to different sites for ease of manipulation. The fragment from 4734 was then placed into the equivalent site of pCGN5143, giving pCGN4735.

A sequence for approximately 3 kb of the promoter construct pCGN4735 is provided in Figure 5. The resynthesized sequence falls between the Nde1 sites located at bases 1706 and 1898 of the sequences. Thus, the sequence in Figure 5 includes approximately 1.7 kb 5′ to the Nde1 site 5′ to the resynthesized polylinker region. There is a roughly 2.5 kb sequence 5′ from this sequence which is not provided in Figure 5, relative to the total 9.2 kb insert. The sequence of Figure 5 also includes approximately 1.1 kb 3′ to the 3′ Nde1 site. Approximately 3 kb which is most 3′ in the Rac13 insert is not provided in Figure 5. A map for pCGN4735 is provided in Figure 6.

Example 7

Constructs for Pigment Synthesis Genes

Constructs which contain encoding sequences for plant or bacterial genes involved in biosynthesis of pigmented compounds, as well as sequences for directing transport of the encoded proteins into plastids or vacuoles are described in copending US patent application to McBride *et al.*, entitled "Use of Ovary Tissue Transcriptional Factors", serial no. 08/480,178 filed on June 7, 1995, the teachings of which are incorporated herein by reference. The targetting sequences are manipulated to be

present on an *Nco*I/*Eco*RI fragment and may easily introduced into the 4-4 and *rac* transcriptional initiation regions for providing transcription in cotton fibers.

Example 8

Cotton Transformation

Explant Preparation

Coker 315 seeds are surface disinfected by placing in 50% Clorox (2.5% sodium hypochlorite solution) for 20 minutes and rinsing 3 times in sterile distilled water. Following surface sterilization, seeds are germinated in 25 x 150 sterile tubes containing 25 mls 1/2 x MS salts: 1/2 x B5 vitamins: 1.5% glucose: 0.3% gelrite. Seedlings are germinated in the dark at 28°C for 7 days. On the seventh day seedlings are placed in the light at 28±2°C.

Cocultivation and Plant Regeneration

Single colonies of A. *tumefaciens* strain 2760 containing binary plasmids pCGN2917 and pCGN2926 are transferred to 5 ml of MG/L broth and grown overnight at 30°C. Bacteria cultures are diluted to 1 x 10^8 cells/ml with MG/L just prior to cocultivation. Hypocotyls are excised from eight day old seedlings, cut into 0.5-0.7 cm sections and placed onto tobacco feeder plates (Horsch *et al.* 1985). Feeder plates are prepared one day before use by plating 1.0 ml tobacco suspension culture onto a petri plate containing Callus Initiation Medium CIM without antibiotics (MS salts: B5 vitamins: 3 % glucose: 0.1 mg/L 2,4-D: 0.1 mg/L kinetin: 0.3% gelrite, pH adjusted to 5.8 prior

to autoclaving). A sterile filter paper disc (Whatman #1) was placed on top of the feeder cells prior to use. After all sections are prepared, each section was dipped into an A. tumefaciens culture, blotted on sterile paper towels and returned to the tobacco feeder plates.

Following two days of cocultivation on the feeder plates, hypocotyl sections are placed on fresh Callus Initiation Medium containing 75 mg/L kanamycin and 500 mg/L carbenicillin. Tissue was incubated at 28±2°C, 30uE 16:8 light:dark period for 4 weeks. At four weeks the entire explant was transferred to fresh callus initiation medium containing antibiotics. After two weeks on the second pass, the callus was removed from the explants and split between Callus Initiation Medium and Regeneration Medium (MS salts: 40mM KNO_3: 10 mM NH_4Cl:B5 vitamins:3% glucose:0.3% gelrite:400 mg/L carb:75 mg/L kanamycin).

Embryogenic callus was identified 2-6 months following initiation and was subcultured onto fresh regeneration medium. Embryos are selected for germination, placed in static liquid Embryo Pulsing Medium (Stewart and Hsu medium: 0.01 mg/l NAA: 0.01 mg/L kinetin: 0.2 mg/L GA3) and incubated overnight at 30°C. The embryos are blotted on paper towels and placed into Magenta boxes containing 40 mls of Stewart and Hsu medium solidified with Gelrite. Germinating embryos are maintained at 28±2°C 50 uE $m^{-2}s^{-1}$ 16:8 photoperiod. Rooted plantlets are transferred to soil and established in the greenhouse.

Cotton growth conditions in growth chambers are as follows: 16 hour photoperiod, temperature of approximately 80-85°, light

intensity of approximately 500μEinsteins. Cotton growth conditions in greenhouses are as follows: 14-16 hour photoperiod with light intensity of at least 400μEinsteins, day temperature 90-95°F, night temperature 70-75°F, relative humidity to approximately 80%.

Plant Analysis

Flowers from greenhouse grown T1 plants are tagged at anthesis in the greenhouse. Squares (cotton flower buds), flowers, bolls etc. are harvested from these plants at various stages of development and assayed for enzyme activity. GUS fluorometric and histochemical assays are performed on hand cut sections as described in co-pending application filed for Martineau *et al.*, *supra.* For fiber color characteristics, plants are visually inspected, or northern or western analysis can be performed, if necessary.

As shown by the above results, expression of a gene of interest can be obtained in cells derived from fiber cells, including tomato fiber and cotton fibers, and expression of genes involved in synthesis of pigments combined with appropriate targeting sequences results in modification of color phenotype in the selected plant tissue.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application are specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail, by way of illustration and example for purposes of clarity and understanding, it will be readily apparent to those of ordinary skill in the art that certain changes and modifications may be made thereto, without departing from the spirit or scope of the appended claims.

CLAIMS

1. A DNA sequence comprising as operably joined components in the direction of transcription, a cotton fiber transcriptional factor and an open reading frame encoding a protein of interest, wherein said transcriptional factor is selected from the 4-4 and the *rac* promoter sequences.

2. The DNA Sequence according to Claim 1, further comprising a transport signal encoding sequence from a plant nuclear-encoded gene.

3. The DNA sequence according to Claim 2, wherein said transport signal encoding sequence comprises a plastid transit peptid.

4. The DNA sequence according to Claim 1, wherein said transport signal encoding sequence encodes a signal peptide which provides for transport across the rough endoplasmic reticulum.

5. The DNA sequence according to Claim 4, wherein said sequence further comprises, 3′ to said open reading frame, a vacuolar localization signal.

6. The DNA sequence of Claim 1 wherein said pigment is melanin or indigo.

7. The DNA sequence of Claim 6 wherein said open reading frame is from a bacterial gene.

8. The DNA sequence of Claim 7 wherein said bacterial gene is selected from the group consisting of ORF438, *tyr*A, anthocyanin R gene, anthocyanin C1 gene, *pig*, and *tna*.

9. A DNA construct comprising a promoter for transcription in a plant cell operably joined to said DNA sequence of Claim 1.

10. The DNA construct of Claim 9 wherein said plant cell is a cotton fiber cell.

11. The DNA construct of Claim 10 wherein said promoter is a tomato 4-4 and rac promoter.

13. A plant cell comprising a DNA construct of Claim 9.

14. A plant comprising a cell of Claim 13.

15. A method of modifying fiber phenotype in a cotton plant, said method comprising:

transforming a plant cell with DNA comprising a construct for expression of a protein in a pigment biosynthesis pathway, wherein said construct comprises as operably joined components:

a transcriptional initiation region functional in cells of said plant tissue,

an open reading frame encoding a protein of interest, and

a transcriptional termination region functional in cells of said plant tissue,

wherein said plant tissue comprises a substrate of said protein; and

growing said plant cell to produce a plant comprising said tissue, wherein said protein reacts with said substrate to produce said pigment.

16. The method of Claim 15 further comprising a transport signal encoding sequence from a plant nuclear-encoded gene.

17. The method of Claim 15 wherein said transport signal encoding sequence encodes a signal peptide which provides for transport across the rough endoplasmic reticulum.

18. The method of Claim 16 wherein said DNA comprises constructs for expression of two proteins in a pigment biosynthesis pathway, wherein each of said constructs comprises components i) through iv), and wherein said two proteins are not encoded by the same gene.

19. The method of Claim 17 wherein said DNA comprises constructs for expression of two proteins in a pigment biosynthesis pathway, wherein each of said constructs comprises components i) through iv), and wherein said two proteins are not encoded by the same gene.

20. The method of Claim 18 or 19 wherein said pigment is melanin and said proteins are encoded by *tyr*A and ORF438.

21. The method of Claim 18 wherein said pigment is indigo and said proteins are *tna* and *pig*.

22. The method of Claim 18 wherein said pigment is anythocyanin and said constructs comprise the anthocyanin R and C1 regulatory genes.

23. The method of Claim 15 wherein plant tissue is a cotton burr.

25. A recombinant DNA construct comprising the cotton tissue transcriptional sequence shown in Figure 2.

26. A recombinant DNA construct comprising the cotton tissue transcriptional sequence shown in Figure 5.

27. An isolated DNA encoding sequence of Figure 1.

28. An isolated DNA encoding sequence of Figure 4.

29. The method of Claim 15 wherein said protein of interest is involved in the synthesis of a plant hormone.

COTTON FIBER TRANSCRIPTIONAL FACTORS

ABSTRACT

Novel DNA constructs are provided which may be used as molecular probes or inserted into a plant host to provide for modification of transcription of a DNA sequence of interest in cotton fiber, particularly in very early fiber development. The DNA constructs comprise a cotton fiber transcriptional initiation regulatory region associated with a gene which is expressed in cotton fiber.

```
                               20                                  40
CTT TCT ATT TGG TTA ACC ATG GCT CAT AAC TTT CGT CAT CCT TTC TTC
Leu Ser Ile Trp Leu Thr Met Ala His Asn Phe Arg His Pro Phe Phe>

                 60                            80
CTT TTC CAA CTT TTA CTC ATT ACT GTC TCA CTA ATG ATC GGT AGC CAC
Leu Phe Gln Leu Leu Leu Ile Thr Val Ser Leu Met Ile Gly Ser His>

  100                                120                                140
   *
ACC GTC TCG TCA GCG GCT CGA CAT TTA TTC CAC ACA CAA ACA ACC TCA
Thr Val Ser Ser Ala Ala Arg His Leu Phe His Thr Gln Thr Thr Ser>

                     160                                180
TCA GAG CTG CCA CAA TTG GCT TCA AAA TAC GAA AAG CAC GAA GAG TCT
Ser Glu Leu Pro Gln Leu Ala Ser Lys Tyr Glu Lys His Glu Glu Ser>

           200                            220                            240
            *
GAA TAC AAA CAG CCA AAA TAT CAT GAA GAG TAC CCA AAA CAT GAG AAG
Glu Tyr Lys Gln Pro Lys Tyr His Glu Glu Tyr Pro Lys His Glu Lys>

                           260                                 280
CCT GAA ATG TAC AAG GAG GAA AAA CAA AAA CCC TGC AAA CAT CAT GAA
Pro Glu Met Tyr Lys Glu Glu Lys Gln Lys Pro Cys Lys His His Glu>

                 300                          320
                  *
GAG TAC CAC GAG TCA CGC GAA TCG AAG GAG CAC GAA GAG TAC GAT AAA
Glu Tyr His Glu Ser Arg Glu Ser Lys Glu His Glu Glu Tyr Asp Lys>

  340                                360                                380
GAA AAA CCC GAT TTC CCC AAA TGG GAA AAG CCT AAA GAG CAC GAG AAA
Glu Lys Pro Asp Phe Pro Lys Trp Glu Lys Pro Lys Glu His Glu Lys>

                     400                                420
```

FIGURE 1A

```
                         *
CAC GAA GTC GAA TAT CCG AAA ATA CCC GAG TAC AAG GAC AAA CAA GAT
His Glu Val Glu Tyr Pro Lys Ile Pro Glu Tyr Lys Asp Lys Gln Asp>

        440                                 460                             480
GAG AAT AAG AAA CAT AAA GAT GAA GAG TGC CAG GAG TCA CAC GAA TCG
Glu Asn Lys Lys His Lys Asp Glu Glu Cys Gln Glu Ser His Glu Ser>

                            500                             520
                             *
AAA GAG CAC GAA GAG TAC GAG AAA GAA AAA CCC GAT TTC CCC AAA TGG
Lys Glu His Glu Glu Tyr Glu Lys Glu Lys Pro Asp Phe Pro Lys Trp>

              540                             560
GAA AAG CCT AAA GGG CAC GAG AAA CAT AAA GCC GAA TAT CCG AAA ATA
Glu Lys Pro Lys Gly His Glu Lys His Lys Ala Glu Tyr Pro Lys Ile>

  580                               600                             620
                                     *
CCT GAG TGC AAG GAA AAA CTA GAT GAG GAT AAG GAA CAT AAA CAT GAG
Pro Glu Cys Lys Glu Lys Leu Asp Glu Asp Lys Glu His Lys His Glu>

                      640                             660
TTC CCA AAG CAT GAA AAA GAA GAG GAG AAG AAA CCT GAG AAA GGC ATA
Phe Pro Lys His Glu Lys Glu Glu Glu Lys Lys Pro Glu Lys Gly Ile>

        680                                 700                             720
                                             *
GTA CCC TGA GTG GGT TAA AAT GCC TGA ATG GCC GAA GTC CAT GTT TAC
Val Pro *** Val Gly *** Asn Ala *** Met Ala Glu Val His Val Tyr>

                            740                             760
TCA GTC TGG CTC GAG CAC TAA GCC TTA AGC CAT ATG ACA CTG GTG CAT
Ser Val Trp Leu Glu His *** Ala Leu Ser His Met Thr Leu Val His>

              780                             800
                                               *
```

FIGURE 1B

```
GTG CCA TCA TCA TGC AGT AAT TTC ATG GGA TAT TGT AAT TAT ATT GTT
Val Pro Ser Ser Cys Ser Asn Phe Met Gly Tyr Cys Asn Tyr Ile Val>

  820                           840                         860
AAT AAA AAA GAT GGT GAG TGG GAA ATG TGT GTG TGC ATT CAT CCA TGA
Asn Lys Lys Asp Gly Glu Trp Glu Met Cys Val Cys Ile His Pro ***>

                    880                           900
                                                    *
GCA ATG CTG AAT CTC TTT GCA TGC ATA GAG ATT CTG AAT GGT TAT AGT
Ala Met Leu Asn Leu Phe Ala Cys Ile Glu Ile Leu Asn Gly Tyr Ser>

        920                           940                     960
TTA TGT TAT ATC GTT TGT TCT AGT GAA ATT AAT TTT GAA TGT TGT ATG
Leu Cys Tyr Ile Val Cys Ser Ser Glu Ile Asn Phe Glu Cys Cys Met>

TAA TGT T
*** Cys Xxx>
```

FIGURE 1C

```
                    20                    40                    60
ACTAAAGGGA ACAAAAGCTG GAGCTCCACC GCGGTGGCGG CCGCTCTAGA ACTAGTGGAT

                    80                   100                   120
                                           *
CCCCCGTGGA CTAAACAAAA CATGGGAAGA TTTGCTGTAA AAAAATAAAA GAAGCTTACT

                   140                   160                   180
CAATAACACT TTGTGAATTG TATACAAAAG ACTCAATGAA AAACAATAAC TCAATACACT

                   200                   220                   240
                     *
TTTTTTCACT GATTTACATC CTTTATATAG GCTGAAACTA CAACAACTTT AGCTAAAAAA

                   260                   280                   300
                                                                 *
ATAGGATAAC CTAATAGCAA AATCACAATC AGATATTAAA CCATGATTTT AGCTAACCAT

                   320                   340                   360
TTAACAACTT TATTGAAACT AATTTGAATA TTTCATCTGC TGATATGCCC AAGATTTTAG

                   380                   400                   420
                                           *
GCCACTAACC GATTTGGTGG TGAACTTTAA CATGTCATGC ATTTGTAACT GTTTGAAACA

                   440                   460                   480
AGTTTTTTGC ATTATTTTAC TATATGAACT GTTTGATTAG GTTGAGTTAC ACACTGAGCT

                   500                   520                   540
                     *
TGTAAGCTCA CTCAAATTTT TCTAATTTCT AAGGTGATCA GCAAACTTAG GACCGGGCGG

                   560                   580                   600
                                                                 *
CGTACGAGAG CTCGGATTGA TTTTCTAGTT AATAAATAAG ACGATTTATG TTTTTAAACT
```

Figure 2A

```
                   620                  640                  660
ATTATGGACT TTTTGGACTA TGTAACTGTT TGGGACTTTA TTTTTGTTTT TTATTTGCTT

                   680                  700                  720
                                          *
TTTTTGGATT TAGTAATTAT TATTTTTAAA CTGCAAAATT ATATGTTTTT ACAAACTAAG

                   740                  760                  780
TCACAGTTTT CAAAATTCCA TAACTTAGAA TTTTTCGCTG CAAAATAAAG TAATCATTTA

                   800                  820                  840
                     *
AGTGTTTTTT CTGTAATAAA ATAAATAAAT AATTTTAACG AGTATTTTCC TAAAAATTGG

                   860                  880                  900
                                                                *
AAATTGATTT ACCAAAATTA GTATGTCAAA ACACATGTTT ATATGTTACA GGGCGATATC

                   920                  940                  960
GTCTAGGCAA ATAACATCTA GGCGGGGTTT GGAGTGTTAC AGGGCGAGTG GGCTCATTTT

                   980                 1000                 1020
                                          *
GAGTAAGTAT AGTTAGGGCC GAGTTTTAGA TTGCATATTC AAGGTCAAAG ATTTTGTAAA

                  1040                 1060                 1080
CTTCGATGAA TGATATGTAT GATTGTCCGA TTAACGAAAT ATGTTTTTTT CTTTTGTGTG

                  1100                 1120                 1140
                     *
TGTTTTATCT CGTGTGATAA GTATATAGTA TGTTTTATTC CAATTCTTAT GGCATGTGAC

                  1160                 1180                 1200
                                                                *
ATTGTGGCTA TTCTAATTAA ATTGATTTGT TATTATTGAA ATCTGATGCA TCTGTTCTAC

                  1220                 1240                 1260
```

Figure 2B

```
AAAGCATGGA ATCTCATGCC TACTGCTTTC TGTTAAAGAT ACGATTGCAA GTTTAACATG

                 1280                  1300                  1320
                                          *
CTTACTATTT TGATTTTGTC CTTGCATGCT ATGTCACATT ACATGGGGTT GGGATGATAT

                 1340                  1360                  1380
GGTAAGGAGG AAGTTTTGAC AGTTTAATGA TTTGCACTAT CTGGTGGTTT AACCACATAT

                 1400                  1420                  1440
                    *
TTGTTATGGC ATCTTGACTG CGGTTATGGT GGCTCGACCG CCCATATCTG TTCTGGAAAT

                 1460                  1480                  1500
                                                                *
TTATCTGTGA CTCTGGTGGC ATTGTCTACA ATTATTTGTT GGTGTGTTTT GGATGGACGA

                 1520                  1540                  1560
GTCGTGGGGA ACTCTATTTG GTGTGTTGCG GAGTTGGGTA GGAAATTTTC GAAAAAAATT

                 1580                  1600                  1620
                                          *
TGCATTGTGT TTTTCTGAAA AATATTGCAT TAACATAATC ATGCATTCTC AATTTTGGTC

                 1640                  1660                  1680
AATTGAACGT TATAAAATTC TCTATGATAT CCTGATCTGT TTATTACATT ATATGTGTTT

                 1700                  1720                  1740
                    *
ATGCTTGAGT TAAGTCAAAC ATTGAGATTC ATAGCTCACC CAATTATTTA ATCATTTCAG

                 1760                  1780                  1800
                                                                *
GCAATCTGCA GACTTAGGAT TGGATGGCGT TCAGGAGCTT GGATTGGTTT TCTCACATCA

                 1820                  1840                  1860
TATTTTATTA AATAATTATT AATTAAAATT TATGGACTTT TGGACTGTCT GACTAATTTT
```

Figure 2C

```
                    1880                 1900                 1920
                                            *
CAGAATTTTA TTTTGGTTTT GGGTTTTGTT GAATTTTTTA GATAATTATT TTAAATATTC

                    1940                 1960                 1980
TGCATAATTT TTCTGTTATT TGAAAAGGAT GTTCGAATTT TTTTTCAAAA TTGAAACGTT

                    2000                 2020                 2040
                       *
TAAGAATTTT TACTACTGCA AATTCAGAAT AAGTGAATTT GTTTTTTAGA AAGATTAAAT

                    2060                 2080                 2100
                                                                 *
AAGTTAGTAT TACGATTTTT AGTTTGATTT GGTGGAAAGT AATGTATGTT TTTGAACATA

                    2120                 2140                 2160
ATTATTTGAC AATAATTAAG TTTTCTAGGG AATAAACGGA AATATCTTCT TCTTTTTTGT

                    2180                 2200                 2220
                                            *
AAAATTACTA ATGCAAGAAC AAACAACGTT TTGGGGAGCA AATAATCTAG CTTTAAGTAG

                    2240                 2260                 2280
TCAGTGTAAC TCTCAAAATC TGGTCATAAC TTCTAGGCTG AGTTTGCTGT GCTACAGTAG

                    2300                 2320                 2340
                       *
TAAGTCTATA GAAACTTACC TGACAAAACG ACATGACGTC AGGGTCGAAT CTACAACTTT

                    2360                 2380                 2400
                                                                 *
TCCTTTTTCT TCAATTAACA TATGGTTGAT TCAAGTTCCG ATCTATAATA ATTTATTACG

                    2420                 2440                 2460
ATTTATCAAT TTCAATTACC TTATATCATC CTATTATAAA TATAAGTCAG TTCAATTCAG
```

Figure 2D

```
                     2480                 2500                 2520
                                             *
TTTTCGAAAG TTCCCAAAAA TTTTGAATTT TATTAAATTT ATTCCCTAAA ACCGAAATAG

                     2540                 2560                 2580
TTATATCTTT CAAATTTAAG TTTCATTTTT CAATCCGATT TCAATTTCAT CCTTTTATAA

                     2600                 2620                 2640
                        *
CTCTCTATTA TCTATAATTA CATAAATTTC AAATTAATTT TGAAATATTT ACACTTTAGT

                     2660                 2680                 2700
                                                                  *
CCCTAAGTTC AAAACTATAA ATTTTCACTT TAGAAATTAA TCATTTTTCA CATCTAAGCA

                     2720                 2740                 2760
TCAAATTTAA CCAAATGACA CAAATTTCAT GATTAGTTAG ATCAAGCTTT TGAGTCTTCA

                     2780                 2800                 2820
                                             *
AAACATAAAA ATTACAAAAA AAAAACAAAC TTAAAATCAT TTATCAATTT GAACAACAAA

                     2840                 2860                 2880
GCTTGGCCGA ATGCTAAGAG CTTAAAAATG GCTTCTTTTG TTTCTTTTTG TTGCAAACGG

                     2900                 2920                 2940
                        *
TGGAGAGAAG AGGGAAATGA AGATTGACCA TATTTTTTTA TTATGTTTTA ACATATAATA

                     2960                 2980                 3000
                                                                  *
TTAATAATTT AATCATAATT ATACTTTGGT GAATGTGACA GTGGGGAGAT ACGTAAAGTA

                     3020                 3040                 3060
TTTTAACATT ATACTTTTTG CAAGCAGTTG GCTGGTCTAC CCAAGAGTGA TCAAAGTTTG

                     3080                 3100                 3120
```

Figure 2E

```
                                                *
AGCTGCCTTC AATGAGCCAA TTTTTGCCCA TAATGGATAA AGGCAATTTG TTTAGTTCAA

                 3140                 3160                 3180
CTGCTCACAG AATAATGTTA AAATGAAATT AAAATAAGGT GGCCTGGTCA CACACACAAA

                 3200                 3220                 3240
                    *
AAAAAACTAA TGTTGGTTGG TTGAATTTTA TATTACGGAA TGTAATATTA TATTTTAAAA

                 3260                 3280                 3300
                                                              *
TAAAATTATG TTATTTAGAT TCTTAATATT TTGGAGCATT CCATACTATA ATTTCGTAAC

                 3320                 3340                 3360
ATAATATTAA AATATAGTAA TATAAAGTGT AATTAACTTT AAATTACAAG CATAATATTA

                 3380                 3400                 3420
                                        *
AATTTTGAAT CAATTAATTT TTATTTCTAT TATTTTAATT AATTTAGTCT ATTTTTTCAA

                 3440                 3460                 3480
AATAAAATTT AAATCTAAAT AAAAATAATT TTTCCTTAAT GTTGAAACAA CTCATGTTAT

                 3500                 3520                 3540
                    *
ACTTCAAAAT TATAAGTATT ATATTTACCT TGATGATTTA TTTATTAGTA TATTAATTCT

                 3560                 3580                 3600
                                                              *
GATTATAATT ATGGTGGGAT ACAATCGCTT TCCACTAAAT ATTTTAACTA TGATTTATAA

                 3620                 3640                 3660
ATTTATTTCA ACATCGTATA TTTACTTATT AATACATAAT TTATCATAAT TTTATGGAAA

                 3680                 3700                 3720
                                        *
```

Figure 2F

```
TTGAGACCAA GAAACATTAA GAGAACAAAT TCTATAACAA AGACAATTTA GAAAAAAATG

                  3740                3760                3780
TACTTTTAGG TAATTTTAAG TACTCTTAAC CAAACACAAA AATTCAAATC AAATGAACTA

                  3800                3820                3840
                     *
AATAAGATAA TATAACATAC GGAACATCTT ACTTGTAATC TTACATTCCC ATAATTTTAT

                  3860                3880                3900
                                                             *
TATGAAAAAT AATCTTATAT TACTCGAACT AAATGTTGTC ACAAATTATT ATCTAAATAA

                  3920                3940                3960
AGAAAAACAC TTAATTTTTA TAACATTTTT TCATATATTT GAAAGATTAT ATTTTGTATA

                  3980                4000                4020
                                         *
TTTACGTAAA AATATTTGAC ATAGATTGAG CACCTTCTTA ACATAATCCC ACCATAAGTC

                  4040                4060                4080
AAGTATGTAG ATGAGAAATT GGTACAAACA ACGTGGGGCC AAATCCCACC AAACCATCTC

                  4100                4120
                     *
TCATTCTCTC CTATAAAAGG CTTGCTACAC ATAGACAACA ATCCACACA C AAA TAC
                                                        <Phe Val

 4140                    4160                          4180
 ACG TTC TTT TCT TTC TAT TTG ATT AAC CAT GGC TCA TAG CAT TCG TCA
<Arg Glu Lys Arg Glu Ile Gln Asn Val Met Ala *** Leu Met Arg ***

                4200                  4220
                   *
 CCC TTT CTT CCT TTT CCA ACT TTT ACT CAT AAG TGT CTC ACT AGT GAC
<Gly Lys Lys Arg Lys Trp Ser Lys Ser Met Leu Thr Glu Ser Thr Val
```

Figure 2G

```
          4240                                     4260                                      4280
 CGG TAG CCA CAC TGT TTC GGC AGC GGC TCG ACG TTT ATT CGA GAC ACA
<Pro Leu Trp Val Thr Glu Ala Ala Ala Arg Arg Lys Asn Ser Val Cys

                             4300                                       4320
                               *
 AGC AAC CTC ATC AGA GCT CCC ACA ATT GGC TTC AAA ATA CGA AAG CAC
<Ala Val Glu Asp Ser Ser Gly Cys Asn Ala Glu Phe Tyr Ser Leu Val

                 4340                                     4360
 GAG AGT CTG AAT ACG AAA AGC CAG AAT ACA AAC AGC CAA AGT ATC ACG
<Leu Thr Gln Ile Arg Phe Ala Leu Ile Cys Val Ala Leu Thr Asp Arg

  4380                                4400                                      4420
                                        *
 AAG AGT ACT CAA AAC TTG AGA AGC CTG AAA TGC AAA AGG AGG AAA AAC
<Leu Thr Ser Leu Val Gln Ser Ala Gln Phe Ala Phe Pro Pro Phe Val

                          4440                                    4460
 AAA AAC CCT GCA AAC AGC ATG AAG AGT ACC ACG AGT CAC ACG AAT CAA
<Phe Val Arg Cys Val Ala His Leu Thr Gly Arg Thr Val Arg Ile Leu

           4480                                    4500                                    4520
                                                     *
 AGG AGC AAA AAG AGT ACG AGA AAG AAA ATC TCGACGAA TTCCCCCGGG
<Pro Ala Phe Leu Thr Arg Ser Leu Phe Asp

                            4540                                    4560                                 4580
 CGTCGACGGC TAGCGAAGAT CTTCGGGCCC GTCGAGCCTT GAATCATATG ACACTGGTGC

                            4600                                    4620                                 4640
                              *
 ATGTGCCATC ATCATGCAGT AATTTCATGG TATATCGTAA TATATAGTTA ATAAAAAAGA

                            4660                                    4680                                 4700
                                                                                                           *
 TGGTGATTGG GAAATGTGTG TGTGCATTCC TCCATGCACT AATGGTGAAT CTCTTTGCAT
```

Figure 2H

```
                       4720                           4740                           4760
ACATAGAAAT TCTAAATGGT TATAGTTTAT GTTATAGTGT ATGTTGTAGT GAAATTAATT

                       4780                           4800                           4820
                                                         *
TTAAATGTTG TATCTAATGT TAACATCACT TGGCTTGATT TATGTTATGT TATGTATTTT

                       4840                           4860                           4880
ACTTTAATGA TATTGCATGT ATTGTTAATT TAACATTGCT TGATCATTAT ACTCTTCTAC

                       4900                           4920                           4940
                          *
TATTAATTAT AAATGGCACT GTTTTGTTTA AACTTTTTAC AAGTTAAGAC ATGTATAAAT

                       4960                           4980                           5000
                                                                                       *
ATATGACAAT ATAATTACAG GTTTTAGTTC AATGTTAGCT ATCTTAGTAT GTTATTGATG

                       5020                           5040                           5060
ATCTTAATTA CATTTAAACA AATTCCACTT AAAATTTTAA TAAATAATAA CAAATAATTA

                       5080                           5100                           5120
                                                         *
TTGTAATATA ATACATTAAA TGCAACAAAA AATGAAATAA ATAAAATAAA ATAGCAAATA

                       5140                           5160                           5180
ATTGTTATAA TATTGTAATA TAATATGTAC CATATTCTTA ACTGAAATAG GGTCTAACCT

                       5200                           5220                           5240
                          *
ATAATCCCTA AAATTTCAGT TTAAATATTT TTATACCTAC CATATTATTA GAACTCTTTT

                       5260                           5280                           5300
                                                                                       *
TAAATATATT AAAATTTTAA TTATACCAAT TTAATTAAAC TATTAATTAT CTTAACTAAA
```

Figure 2I

```
                     5320                 5340                 5360
ATCTAAAATT TTATTTAACC TATTAATAAA TTCCTAATTA TCTTATCTAA TTTAAAACTC

                     5380                 5400                 5420
                                             *
TAATTATCCT AATTTAATTT AAATTCTTAA TTATCTTAAT TTGTAACCTC CTCCACCCAG

                     5440                 5460                 5480
CTAGATGCTG GACCCGAATC CGGGAGATTA CATCGGCCAT TGAGATGGCG TGATCAGGGT

                     5500                 5520                 5540
                        *
TTGGCGCGCC GGTACCCAAT TCGCCCTATA GTGAGTTCGT ATTACGCGCG CTCACTGCGT

CCGGTTT
```

Figure 2J

```
                    20                   40                   60
ACTAAAGGGA ACAAAAGCTG GAGCTCCACC GCGGTGGCGG CCGCTCTAGG ATCCCCCGTG

                    80                  100                  120
                                          *
GACTAAACAA AACATGGGAA GATTTGCTGT AAAAAAATAA AAGAAGCTTA CTCAATAACA

                   140                  160                  180
CTTTGTGAAT TGTATACAAA AGACTCAATG AAAAACAATA ACTCAATACA CTTTTTTTCA

                   200                  220                  240
                     *
CTGATTTACA TCCTTTATAT AGGCTGAAAC TACAACAACT TTAGCTAAAA AAATAGGATA

                   260                  280                  300
                                                                *
ACCTAATAGC AAAATCACAA TCAGATATTA AACCATGATT TTAGCTAACC ATTTAACAAC

                   320                  340                  360
TTTATTGAAA CTAATTTGAA TATTTCATCT GCTGATATGC CCAAGATTTT AGGCCACTAA

                   380                  400                  420
                                          *
CCGATTTGGT GGTGAACTTT AACATGTCAT GCATTTGTAA CTGTTTGAAA CAAGTTTTTT

                   440                  460                  480
GCATTATTTT ACTATATGAA CTGTTTGATT AGGTTGAGTT ACACACTGAG CTTGTAAGCT

                   500                  520                  540
                     *
CACTCAAATT TTTCTAATTT CTAAGGTGAT CAGCAAACTT AGGACCGGGC GGCGTACGAG

                   560                  580                  600
                                                                *
AGCTCGGATT GATTTTCTAG TTAATAAATA AGACGATTTA TGTTTTTAAA CTATTATGGA
```

Figure 3A

```
                      620                                    640                                    660
CTTTTTGGAC TATGTAACTG TTTGGGACTT TATTTTTGTT TTTTATTTGC TTTTTTTGGA

                      680                                    700                                    720
                                                               *
TTTAGTAATT ATTATTTTTA AACTGCAAAA TTATATGTTT TTACAAACTA AGTCACAGTT

                      740                                    760                                    780
TTCAAAATTC CATAACTTAG AATTTTTCGC TGCAAAATAA AGTAATCATT TAAGTGTTTT

                      800                                    820                                    840
                        *
TTCTGTAATA AAATAAATAA ATAATTTTAA CGAGTATTTT CCTAAAAATT GGAAATTGAT

                      860                                    880                                    900
                                                                                                      *
TTACCAAAAT TAGTATGTCA AAACACATGT TTATATGTTA CAGGGCGATA TCGTCTAGGC

                      920                                    940                                    960
AAATAACATC TAGGCGGGGT TTGGAGTGTT ACAGGGCGAG TGGGCTCATT TTGAGTAAGT

                      980                                   1000                                   1020
                                                              *
ATAGTTAGGG CCGAGTTTTA GATTGCATAT TCAAGGTCAA AGATTTTGTA AACTTCGATG

                     1040                                   1060                                   1080
AATGATATGT ATGATTGTCC GATTAACGAA ATATGTTTTT TTCTTTTGTG TGTGTTTTAT

                     1100                                   1120                                   1140
                        *
CTCGTGTGAT AAGTATATAG TATGTTTTAT TCCAATTCTT ATGGCATGTG ACATTGTGGC

                     1160                                   1180                                   1200
                                                                                                      *
TATTCTAATT AAATTGATTT GTTATTATTG AAATCTGATG CATCTGTTCT ACAAAGCATG

                     1220                                   1240                                   1260
```

Figure 3B

```
GAATCTCATG CCTACTGCTT TCTGTTAAAG ATACGATTGC AAGTTTAACA TGCTTACTAT

                 1280                    1300                    1320
                                            *
TTTGATTTTG TCCTTGCATG CTATGTCACA TTACATGGGG TTGGGATGAT ATGGTAAGGA

                 1340                    1360                    1380
GGAAGTTTTG ACAGTTTAAT GATTTGCACT ATCTGGTGGT TTAACCACAT ATTTGTTATG

                 1400                    1420                    1440
                    *
GCATCTTGAC TGCGGTTATG GTGGCTCGAC CGCCCATATC TGTTCTGGAA ATTTATCTGT

                 1460                    1480                    1500
                                                                    *
GACTCTGGTG GCATTGTCTA CAATTATTTG TTGGTGTGTT TTGGATGGAC GAGTCGTGGG

                 1520                    1540                    1560
GAACTCTATT TGGTGTGTTG CGGAGTTGGG TAGGAAATTT TCGAAAAAAA TTTGCATTGT

                 1580                    1600                    1620
                                            *
GTTTTTCTGA AAAATATTGC ATTAACATAA TCATGCATTC TCAATTTTGG TCAATTGAAC

                 1640                    1660                    1680
GTTATAAAAT TCTCTATGAT ATCCTGATCT GTTTATTACA TTATATGTGT TTATGCTTGA

                 1700                    1720                    1740
                    *
GTTAAGTCAA ACATTGAGAT TCATAGCTCA CCCAATTATT TAATCATTTC AGGCAATCTG

                 1760                    1780                    1800
                                                                    *
CAGACTTAGG ATTGGATGGC GTTCAGGAGC TTGGATTGGT TTTCTCACAT CATATTTTAT

                 1820                    1840                    1860
TAAATAATTA TTAATTAAAA TTTATGGACT TTTGGACTGT CTGACTAATT TTCAGAATTT
```

Figure 3C

```
                        1880                 1900                 1920
                                                *
TATTTTGGTT TTGGGTTTTG TTGAATTTTT TAGATAATTA TTTTAAATAT TCTGCATAAT

                        1940                 1960                 1980
TTTTCTGTTA TTTGAAAAGG ATGTTCGAAT TTTTTTTCAA AATTGAAACG TTTAAGAATT

                        2000                 2020                 2040
                           *
TTTACTACTG CAAATTCAGA ATAAGTGAAT TTGTTTTTTA GAAAGATTAA ATAAGTTAGT

                        2060                 2080                 2100
                                                                     *
ATTACGATTT TTAGTTTGAT TTGGTGGAAA GTAATGTATG TTTTTGAACA TAATTATTTG

                        2120                 2140                 2160
ACAATAATTA AGTTTTCTAG GGAATAAACG GAAATATCTT CTTCTTTTTT GTAAAATTAC

                        2180                 2200                 2220
                                                *
TAATGCAAGA ACAAACAACG TTTTGGGGAG CAAATAATCT AGCTTTAAGT AGTCAGTGTA

                        2240                 2260                 2280
ACTCTCAAAA TCTGGTCATA ACTTCTAGGC TGAGTTTGCT GTGCTACAGT AGTAAGTCTA

                        2300                 2320                 2340
                           *
TAGAAACTTA CCTGACAAAA CGACATGACG TCAGGGTCGA ATCTACAACT TTTCCTTTTT

                        2360                 2380                 2400
                                                                     *
CTTCAATTAA CATATGGTTG ATTCAAGTTC CGATCTATAA TAATTTATTA CGATTTATCA

                        2420                 2440                 2460
ATTTCAATTA CCTTATATCA TCCTATTATA AATATAAGTC AGTTCAATTC AGTTTTCGAA
```

Figure 3D

```
                      2480                   2500                   2520
                                                *
AGTTCCCAAA AATTTTGAAT TTTATTAAAT TTATTCCCTA AAACCGAAAT AGTTATATCT

                      2540                   2560                   2580
TTCAAATTTA AGTTTCATTT TTCAATCCGA TTTCAATTTC ATCCTTTTAT AACTCTCTAT

                      2600                   2620                   2640
                         *
TATCTATAAT TACATAAATT TCAAATTAAT TTTGAAATAT TTACACTTTA GTCCCTAAGT

                      2660                   2680                   2700
                                                                       *
TCAAAACTAT AAATTTTCAC TTTAGAAATT AATCATTTTT CACATCTAAG CATCAAATTT

                      2720                   2740                   2760
AACCAAATGA CACAAATTTC ATGATTAGTT AGATCAAGCT TTTGAGTCTT CAAAACATAA

                      2780                   2800                   2820
                                                *
AAATTACAAA AAAAAAACAA ACTTAAAATC ATTTATCAAT TTGAACAACA AAGCTTGGCC

                      2840                   2860                   2880
GAATGCTAAG AGCTTAAAAA TGGCTTCTTT TGTTTCTTTT TGTTGCAAAC GGTGGAGAGA

                      2900                   2920                   2940
                         *
AGAGGGAAAT GAAGATTGAC CATATTTTTT TATTATGTTT TAACATATAA TATTAATAAT

                      2960                   2980                   3000
                                                                       *
TTAATCATAA TTATACTTTG GTGAATGTGA CAGTGGGGAG ATACGTAAAG TATTTTAACA

                      3020                   3040                   3060
TTATACTTTT TGCAAGCAGT TGGCTGGTCT ACCCAAGAGT GATCAAAGTT TGAGCTGCCT

                      3080                   3100                   3120
```

Figure 3E

```
                                          *
TCAATGAGCC AATTTTTGCC CATAATGGAT AAAGGCAATT TGTTTAGTTC AACTGCTCAC

                 3140                 3160                 3180
AGAATAATGT TAAAATGAAA TTAAAATAAG GTGGCCTGGT CACACACACA AAAAAAAACT

                 3200                 3220                 3240
                    *
AATGTTGGTT GGTTGAATTT TATATTACGG AATGTAATAT TATATTTTAA AATAAAATTA

                 3260                 3280                 3300
                                                              *
TGTTATTTAG ATTCTTAATA TTTTGGAGCA TTCCATACTA TAATTTCGTA ACATAATATT

                 3320                 3340                 3360
AAAATATAGT AATATAAAGT GTAATTAACT TTAAATTACA AGCATAATAT TAAATTTTGA

                 3380                 3400                 3420
                                          *
ATCAATTAAT TTTTATTTCT ATTATTTTAA TTAATTTAGT CTATTTTTTC AAAATAAAAT

                 3440                 3460                 3480
TTAAATCTAA ATAAAAATAA TTTTTCCTTA ATGTTGAAAC AACTCATGTT ATACTTCAAA

                 3500                 3520                 3540
                    *
ATTATAAGTA TTATATTTAC CTTGATGATT TATTTATTAG TATATTAATT CTGATTATAA

                 3560                 3580                 3600
                                                              *
TTATGGTGGG ATACAATCGC TTTCCACTAA ATATTTTAAC TATGATTTAT AAATTTATTT

                 3620                 3640                 3660
CAACATCGTA TATTTACTTA TTAATACATA ATTTATCATA ATTTTATGGA AATTGAGACC

                 3680                 3700                 3720
                                          *
```

Figure 3F

```
AAGAAACATT AAGAGAACAA ATTCTATAAC AAAGACAATT TAGAAAAAAA TGTACTTTTA

                     3740                   3760                   3780
GGTAATTTTA AGTACTCTTA ACCAAACACA AAAATTCAAA TCAAATGAAC TAAATAAGAT

                     3800                   3820                   3840
                        *
AATATAACAT ACGGAACATC TTACTTGTAA TCTTACATTC CCATAATTTT ATTATGAAAA

                     3860                   3880                   3900
                                                                      *
ATAATCTTAT ATTACTCGAA CTAAATGTTG TCACAAATTA TTATCTAAAT AAAGAAAAAC

                     3920                   3940                   3960
ACTTAATTTT TATAACATTT TTTCATATAT TTGAAAGATT ATATTTTGTA TATTTACGTA

                     3980                   4000                   4020
                                               *
AAAATATTTG ACATAGATTG AGCACCTTCT TAACATAATC CCACCATAAG TCAAGTATGT

                     4040                   4060                   4080
AGATGAGAAA TTGGTACAAA CAACGTGGGG CCAAATCCCA CCAAACCATC TCTCATTCTC

                     4100                   4120
                        *
TCCTATAAAA GGCTTGCTAC ACATAGACAA CAATCCACAC A CA AAT ACA CGT TCT
                                                <Ile Cys Thr Arg

   4140                     4160                 4180
 TTT CTT TCT ATT TGA TTA ACC ATG G CTCATAGCAT TCGTCACCCT TTCTTCCTTT
<Lys Lys Arg Asn Ser *** Gly His

        4200                     4220                   4240
          *
TCCAACTTTT ACTCATAAGT GTCTCACTAG TGACCGGTAG CCACACTGTT TCGGCAGCGG

        4260                     4280                   4300
```

Figure 3G

```
                                                            *
CTCGACGTTT ATTCGAGACA CAAGCAACCT CATCAGAGCT CCCACAATTG GCTTCAAAAT
      4320                  4340                  4360
ACGAAAAGCA CGAAGAGTCT GAATACGAAA AGCCAGAATA CAAACAGCCA AAGTATCACG

      4380                  4400                  4420
                               *
AAGAGTACTC AAAACTTGAG AAGCCTGAAA TGCAAAAGGA GGAAAAACAA AAACCCTGCA
      4440                  4460                  4480
AACAGCATGA AGAGTACCAC GAGTCACACG AATCAAAGGA GCAAAAAGAG TACGAGAAAG

      4500                  4520                  4540
         *
AAAATCTCGA CGGGCCCGAA GATCTTCGCT AGCCGTCGAC GCCCGGGGGA ATTCGTCGAG

      4560                  4580                  4600
                                                     *
CCTTGAATCA TATGACGCTG GTGCATGTGC CATCATCATG CAGTAATTTC ATGGTATATC
      4620                  4640                  4660
GTAATATATA GTTAATAAAA AAGATGGTGA TTGGGAAATG TGTGTGTGCA TTCCTCCATG

      4680                  4700                  4720
                               *
CACTAATGGT GAATCTCTTT GCATACATAG AAATTCTAAA TGGTTATAGT TTATGTTATA
      4740                  4760                  4780
GTGTATGTTG TAGTGAAAKT AATTTTAAAT GTTGTATCTA ATGTTAACAT CACTTGGCTT

      4800                  4820                  4840
         *
GATTTATGTT ATGTTATGTA TTTTACTTTA ATGATATTGC ATGTATTGTT AATTTAACAT

      4860                  4880                  4900
                                                     *
```

Figure 3H

```
TGCTTGATCA TTATACTCTT CTACTATTAA TTATAAATGG CACTGTTTTG TTTAAACTTT
      4920                 4940                 4960
TTACAAGTTA AGACATGTAT AAATATATGA CAATATAATT ACAAGTTTTA GTTCAATGTT
      4980                 5000                 5020
                              *
AGCTATCTTA GTATGTTATT GATGATCTTA ATTACATTTA AACAAATTCC ACTTAAAATT
      5040                 5060                 5080
TTAATAAATA ATAACAAATA ATTATTGTAA TATAATACAT TAAATGCAAC AAAAAATGAA
      5100                 5120                 5140
         *
ATAAATAAAA TAAAATAGCA AATAATTGTT ATAATATTGT AATATAATAT GTACCATATT
      5160                 5180                 5200
                                                    *
CTTAACTGAA ATAGGGTCTA ACCTATAATC CCTAAAATTT CAGTTTAAAT ATTTTTATAC
      5220                 5240                 5260
CTGCCATATT ATTAGAACTC TTTTTAAATA TATTAAAATT TTAATTATAC CAATTTAATT
      5280                 5300                 5320
                              *
TAAACTATTA ATTATCTTAA CTAAAATCTA AAATTTTATT TAACCTATTA ATTAAATTCC
      5340                 5360                 5380
TAATTATCTT ATCTAATTTA AAACTCTAAT TATCCTAATT TGATTTAAAT TCTTGATTAT
      5400                 5420                 5440
         *
CTTAATTTGT AACCTCCTCC ACCCAGCTAG ATGCTGGACC CGAATCCGGG AGATTACATC
      5460                 5480                 5500
                                                    *
GGCATTGAGA TGGCCTAGTA GTGATCAGGG TTTTCTAGAG GTACCCAATT CGCCCTATAG
```

Figure 3I

TGAGTCGT

Figure 3J

```
AAAAAACA ATG AGC ACT GCA AGA TTT ATC AAG TGT GTC ACG GTC GGT GAT     50
         Met Ser Thr Ala Arg Phe Ile Lys Cys Val Thr Val Gly Asp
          1               5                  10

GGA GCT GTG GGG AAA ACT TGT ATG CTC ATT TCA TAT ACC AGC AAT ACT      98
Gly Ala Val Gly Lys Thr Cys Met Leu Ile Ser Tyr Thr Ser Asn Thr
 15                  20                  25                  30

TTC CCA ACG GAT TAT GTT CCA ACA GTA TTT GAT AAC TTT AGT GCC AAT     146
Phe Pro Thr Asp Tyr Val Pro Thr Val Phe Asp Asn Phe Ser Ala Asn
                 35                  40                  45

GTG GTG GTG GAT GGC AGC ACA GTG AAC CTT GGC CTA TGG GAC ACT GCC     194
Val Val Val Asp Gly Ser Thr Val Asn Leu Gly Leu Trp Asp Thr Ala
             50                  55                  60

GGG CAA GAA GAT TAT AAT AGG CTA AGG CCA CTG AGT TAT AGA GGA GCT     242
Gly Gln Glu Asp Tyr Asn Arg Leu Arg Pro Leu Ser Tyr Arg Gly Ala
         65                  70                  75

GAT GTG TTT TTG TTG GCC TTT TCT CTT ATA AGC AAG GCC AGT TAT GAA     290
Asp Val Phe Leu Leu Ala Phe Ser Leu Ile Ser Lys Ala Ser Tyr Glu
     80                  85                  90

AAC ATC TAC AAA AAG TGG ATC CCA GAG CTA AGA CAT TAT GCT CAT AAT     338
Asn Ile Tyr Lys Lys Trp Ile Pro Glu Leu Arg His Tyr Ala His Asn
 95                 100                 105                 110

GTA CCA GTT GTG CTT GTT GGA ACC AAA CTA GAT TTG CGA GAT GAC AAG     386
Val Pro Val Val Leu Val Gly Thr Lys Leu Asp Leu Arg Asp Asp Lys
                115                 120                 125

CAG TTC CTC ATT GAT CAC CCT GGA GCA ACA CCA ATA TCA ACA TCT CAG     434
Gln Phe Leu Ile Asp His Pro Gly Ala Thr Pro Ile Ser Thr Ser Gln
            130                 135                 140

GGA GAA GAA CTA AAG AAG ATG ATA GGA GCA GTT ACT TAT ATA GAA TGC     482
```

FIGURE 4A

```
Gly Glu Glu Leu Lys Lys Met Ile Gly Ala Val Thr Tyr Ile Glu Cys
        145                 150                 155

AGC TCC AAA ACC CAA CAG AAT GTG AAG GCT GTT TTC GAT GCT GCA ATA     530
Ser Ser Lys Thr Gln Gln Asn Val Lys Ala Val Phe Asp Ala Ala Ile
    160                 165                 170

AAA GTA GCT TTG AGG CCA CCA AAA CCA AAG AGA AAG CCT TGC AAA AGG     578
Lys Val Ala Leu Arg Pro Pro Lys Pro Lys Arg Lys Pro Cys Lys Arg
175                 180                 185                 190

AGA ACA TGT GCT TTC CTT TGAATATTGG ATCATTATTA CAGTCAAAAA            626
Arg Thr Cys Ala Phe Leu
                195

CAGTTAACAA AAGCTGTTGC AGATAAACAC TGAATCTGCT ATAGTTTGTT TTTGGTTTAC   686

ATATGTTCCA CGTGAAACTA TGAAGCATCT CTAAGAAAAC CCAAACTATC ATATCAACCC   746

ATCGATCAAT GAATCGATTT CAATTTTCGC AGTATAAGTT CCTTTTAATC CTTTCTTTTT   806

ACTTCATTTT ATAACGAATT CTATGGATAA TGTTCCCTAC AAACATGTCA TTACAATGTT   866

TAATTATAAA TTCCATTCTT CTATTTTACT AAAAAAAAAA AAAA                    910
```

FIGURE 4B

```
                                20                    40                    60
TTGGATGAGA ACCAATTTTT AATAGTAAAN CCTAACCAAT TTTTAATAAT AAAGCTGACT

                                80                   100                   120
                                                       *
CCTAGTACAA GAGCTTTTAT TCATTCTTCT ATTTTGCTTT CCTCTAGGCT TGGCAATCGA

                               140                   160                   180
GAATTTTCTT GTGTTACAAT ATAATAAATA CATCGTAGAA ATAAATTTTA TTCAAATTGA

                               200                   220                   240
                                 *
AGTCTTAACC ATCTTTAATA TTTGTAGATG TAATTTAAAT GAAAGATAAA TACATATTCT

                               260                   280                   300
                                                                             *
TGGACATGTA TTTTCATCTT AATGTTTGTG GCTTTGGTGA TAGGTGTATT GATGTACGAT

                               320                   340                   360
GTCTTTTAAA TCACATATCA CATTTTGAGT TTGTATGATG ATAAGTCGAC ATAANCGAAA

                               380                   400                   420
                                                       *
TATGGTGTGA TCTTCACTTT TGAACTTTGA TAAGTCACCA AACTTTAACA AAGTTTGATT

                               440                   460                   480
GTGTACATAT ATATATATAT CTTCAAATTT TATAATAAAA ATTGTGTTTA AATAATTTAC

                               500                   520                   540
                                 *
AGTTATATTA TTTTTTTATC TCTAATTTTA TTTGTCGCCA AATTTTTAGT TGATATTTTA

                               560                   580                   600
                                                                             *
ACATAAAAAA AATTGTACAC ATTTACAAGC CCATATACAA ATAATTATAT AAATATTCAT
```

FIGURE 5/A

```
                  620                  640                  660
TAAAAAATAT ATTTAAATAT AGGATATAAA TATAACTATT TTAGAATTAT TCTACTTTAA

                  680                  700                  720
                                         *
GATAACATAG GTTAAATGTA TAATTAATAA GGTTAGTTTA TTGTAAAGAT GAGTATATAT

                  740                  760                  780
GTCGTAAACA TAATCACTAA CCATTTTTAT TAACTTCTTG GTTTTGAAGT TCCAAAAAGA

                  800                  820                  840
                    *
AAATGGAAGG GAAATTTGAG AGTAAGTTCA TGTTTATATT ATACATAATG AAGTTGATGT

                  860                  880                  900
                                                              *
TTTCTTCTTT TTAATATTTT TATACAAAAT ATTTAAATAA AATAATTAAG GATTGAATGA

                  920                  940                  960
AAAATATAAT GAAAGTCGTT TTACTAATAG TCATATTGCA TTTTGTCGCA TCTACTTAAA

                  980                 1000                 1020
                                         *
TAATAGATAA ATTAATTGTG GTACATTAGA TCAAAGAACA AACTAGATTT TGTCCCATTC

                 1040                 1060                 1080
TATTGTTAAA AGCTGGTCCG TTTACATTAA AATAAGGTAC ATGTTACATG CCACGTATAA

                 1100                 1120                 1140
                    *
CTATCTGGTT ATTCTATCAA TCACGGTAAT TTTTAACAGT AGAAATGAAT GTAATTTTTA

                 1160                 1180                 1200
                                                              *
AATAGAAAGG GTCAAATTGT TATTTGATCT AACACGTAGG GATTAATTTA CTTATTTTCC

                 1220                 1240                 1260
```

FIGURE 5/B

```
TAAAGAAATA AGTAAAATAT AATTTGAATC TTAATACAAA AACTTTCATG ATACTTTTAT

                  1280                  1300                  1320
                                           *
CATATTTTAC TTATAATTTA ATATTGTGAG AGTAACAAAR TTAAAAAACA TAGAAACACC

                  1340                  1360                  1380
AAAAGTTAGT TATGGTGTGA CTCATATACA CAGTTAAAAT TTGAATAAAT TTTTTTCTTC

                  1400                  1420                  1440
                     *
GTCATTAATT CCATCATGGG TTTTTTTTTT TCTAGTTAAG CCATAATTAT CAAAATAATC

                  1460                  1480                  1500
                                                                 *
ATCATTAATC CTATCAATAC CCCGCCCTGC CTCCCTCCCT CAATACTTAA ACCCAACTAA

                  1520                  1540                  1560
CACCCAGCAC CAAACGCACT TTAATAGCCA CCTATTTCTA GCCATGTCCT TGCACTTAAA

                  1580                  1600                  1620
                                           *
GAAAAGTAAA GCTAACCTGC AATCATTCCA TATCGAGGCC TCAACAGATA AAGTTGGTTG

                  1640                  1660                  1680
ATGGGTTTGC ACCAAGTTGT TAAAACCCGG CCCTCAACTT CCCTTTTCTT TTCATCCTCC

                  1700                  1720                  1740
                     *
CCACTCCACA CCCTCCAATT TTCTTCATAT GGTTCTATTA TAAGTTCTTT ATAATCACAG

                  1760                  1780                  1800
                                                                 *
AATCAAGATA AGTCCTCAGC AAACAAAAAA CCATGGCTCT CGAGCAAGAT CTGGACTAGT

                  1820                  1840                  1860
CAGAGCTCTG AATATTGGAT CATTATTACA GTCAAAAACA GTTAACAAAA GCTGTTGCAG
```

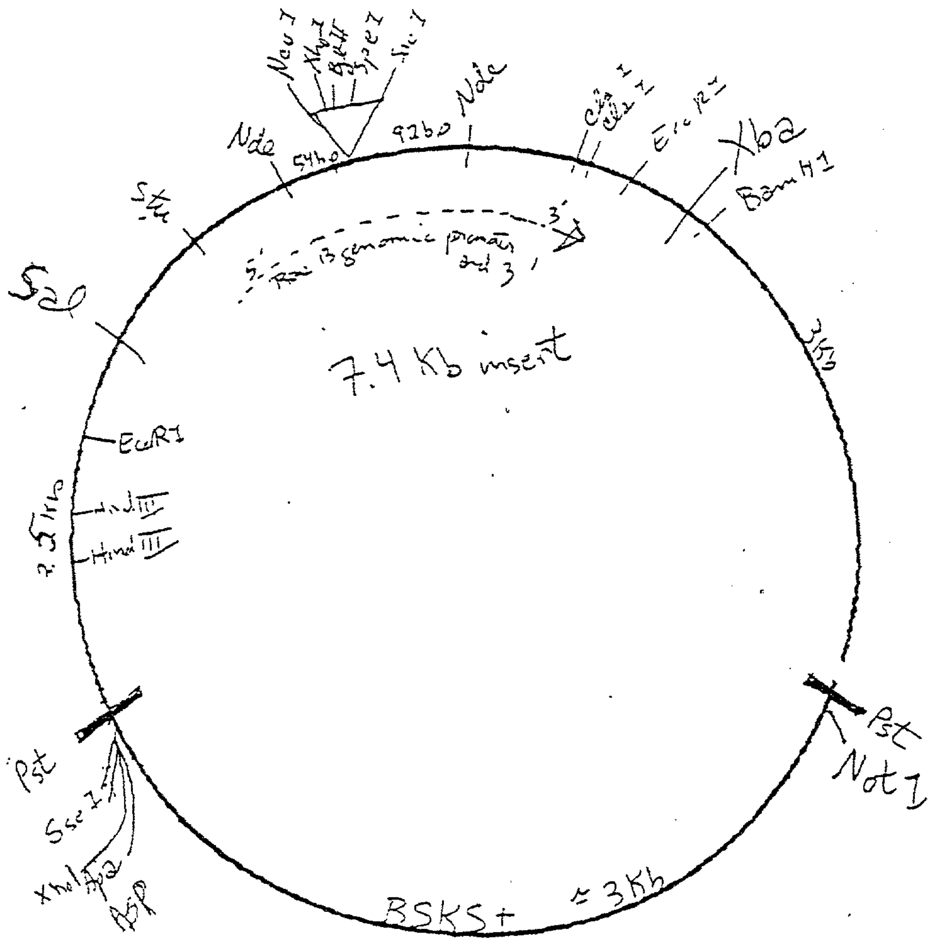
FIGURE 5/C

```
                    1880                 1900                 1920
                                            *
ATAAACACTG AATCTGCTAT AGTTTGTTTT TGGTTTACAT ATGTTCCACG TGAAACTATG

                    1940                 1960                 1980
AAGCATCTCT AAGAAAACCC AAACTATCAT ATCAACCCAT CGATCAATGA ATCGATTTCA

                    2000                 2020                 2040
                       *
ATTTTCGCAG TATAAGTTCC TTTTAATCCT TTCTTTTTAC TTCATTTTAT AACGAATTCT

                    2060                 2080                 2100
                                                                 *
ATGGATAATG TTCCCTACAA ACATGTCATT ACAATGTTTA ATTATAAATT CCATTCTTCT

                    2120                 2140                 2160
ATTTTACTAA GATATTAGTA ACTTCAAACT GCTGATTTTT ACTAATTTAT TATTTATAAA

                    2180                 2200                 2220
                                            *
TTGTTAGAAT GATTATTTTT CAATAATTTA ACAACAATAT TTAATATTAT TATTATTATT

                    2240                 2260                 2280
ATTTCTCAAT TTTTATTAAA CAAAAACATA AATTTTTGAC AAATTAAAAT AAATGAATTA

                    2300                 2320                 2340
                       *
ATTTCTCAAT TTTTCGTGCA ACTATTACAA AAATCCTTCA TAGTCCTAAT CTTAATTTGA

                    2360                 2380                 2400
                                                                 *
TGCAGAGGTG ATAATAATCT TAATTTGATG CAGAGGTAAT AATGGGCCGG GTTTGAGCTG

                    2420                 2440                 2460
GACTTAAGCA TGATATTGAC GTACTTTATA TTTTTCCAAA TTCAACCCAG CTCGAAATAT
```

FIGURE 5/D

```
                    2480                 2500                 2520
                                            *
GAGTCTAAAA TTTTGTCCAA TTTAATCCAA GCCCATTTTA AGTTCGTCCA TATTATTTTT

                    2540                 2560                 2580
TAATTTAAAA AATTTATATC ATTTTATTTT AATATTTAAT TATTTTATAT ATTTTTTATT

                    2600                 2620                 2640
                       *
TATTGAAAAT TTTTATATAG TCATCTTAAC ATTATGTTAA TGTTTATATT AGAGTAGTAT

                    2660                 2680                 2700
                                                                 *
TATATATATT TAGTATAGGT TTATTTTGTT AATAAACTTA AAAATGGGTC TTGTGGGCTA

                    2720                 2740                 2760
GACTTGGACC TTAAATGCTC AAACTCAAAC TTAATTCATA TTTTAAACAG GCTTAATATT

                    2780                 2800                 2820
                                            *
TTTATTTACA CTGTTTCAAA TTTTTCGGGT GAAATATCTT CGAGTCTAGA TTAATAACAC

                    2840                 2860                 2880
CACAGGTCTA ATTTGATGCT CAATGAAAAT GAAATCATAT TGAGCTTAAT TAATATTCCA

                    2900                 2920                 2940
                       *
TTCTTCTTTG CTGAAAGGAC CAAGCAATTC GAGTTACATT AAGGTTAAAG AGTATGGGAT

                    2960                 2980                 3000
                                                                 *
CCGCCAAACC TGCCCCAATG TCTCTTCAAC CATCCAAAAA CTTGAGTCAG TATCACATAC

                    3020                 3040
ATGTACCGNT ATTTATTTAT TTATTGAAAT TGGCATTATT TCTTG
```

FIGURE 5/E



Nde
Nde
Sal
EcoRI
Hind III
Hind III
Pst
Pst
Not I
Xba
Bam H1
7.4 Kb insert
BSKS+
≈ 3Kb
3Kb
5'
3'

FIGURE 6

```
GGGCATTCCA CACGACCATG TGTCCCCTAT TTCCAGGCAT TTTGAGACTT CACCTAAACT   60
TCTAGAGTTG TTTCAAATTA GCCCCTATTT GTTCTTAAAT CATTTTAGGA TCTTGTAAAC  120
TCGTATTTAG GACTAAATGT GTAATTTATA CTTTAATTAT GATTGATTAA TTGATTGATT  180
TNGTAGTAAT GCCCGTGACC CTAATCCGTT AGCGAAGAGG GGTTAGGGGT TAGGGGTTTT  240
ATTATTATTT TTTAGATATT GTATAACTCT TGTTTTATTT TTAATTTTGT TACTATTTCA  300
AAGGCATTTG TTTGTAGTGT TATTTCGAGT AGGTTTTATG GGTGAACAAC CCTTGACCGC  360
CAAATCAATC ACAAGAGTTC AACATTTTAT TTATTTTGAA ATGTATTAAA AATCGTTAAT  420
CTATATATTC GCCCCATTAT TGGGATTAAA TATTCACAAG GGTTTAGACC GTCATGAGAC  480
AGATTAGTTT TATCTTACTG ATGGTCACAT CACAATAGTA ATTCAACTTA ATACGAGAGG  540
AACCATTGAT TCACGCAATT GGTCATCGCA CTTAGTTGAA AAGCTAGGGG TGCGAAGCTA  600
CCGTACGCTG GATTATGATT GAACACCTCT AAGTCAGAAT CCGAATTAGA AACAATGCAC  660
GTGTCCGTTG CCTGATTGCC AACCCCAATA ACACGTGTTG TAGGTTTAAC CATGTTTATG  720
AAAGATAAGG TTTTTTTTTT TATAAGCAAG CAACTATAGG GGTTTACTTC CGTGCGCAAA  780
TTTTTAGGTT ACCTATTTTG GGAGGGGGGA TTATGATTCA AGTGAAAGAA AGTTGGCACA  840
CACACAATCA GTACATCTGT TTTGACAGAG ACACAGCCTA AAAACAGCAG CAAACAAGCC  900
TAAAGGAATC ACCCAAAAAC AACAACCAAA AGTACAGAGG AAAACAAAAG AATCCCTGTT  960
ACCACCAAGC TGAAAAAAAG AAAATAAAAC TCAACTTTTG GCAATAAAAA CCCTCCTACC 1020
CTCAACCCCT AACCACGCAA CAATCAGCAA TACTCCAAGC AACCATTTTC CTTACAAGTT 1080
```

FIGURE 7A

```
TGTTTTTCTT GTGATTAATC CAT ATG GCT AGC TCC ATG TCC CTT AAG CTT GCA 1133
                          Met Ala Ser Ser Met Ser Leu Lys Leu Ala>

TGT CTG CTA GTG TTG TGC ATG GTG GTG GGT GCA CCC CTG GCT CAA GGG   1181
Cys Leu Leu Val Leu Cys Met Val Val Gly Ala Pro Leu Ala Gln Gly>

GAC GTA ACC CGT GCT GAT GGC GTA GTC ACC CTT CCA CGC TGC CTT CCT   1229
Asp Val Thr Arg Ala Asp Gly Val Val Thr Leu Pro Arg Cys Leu Pro>

TTA TTG ATA GGG AAT GGT AAT GGT GCT GAT GCT GAT GTT GAT GCC CCA   1277
Leu Leu Ile Gly Asn Gly Asn Gly Ala Asp Ala Asp Val Asp Ala Pro>

GCT TGC TGC GAC ATC GTC AGG GGT CTC TTG AGC TCG CTG CTC TGT GGT   1325
Ala Cys Cys Asp Ile Val Arg Gly Leu Leu Ser Ser Leu Leu Cys Gly>

GGT GTT TAGGAACCG ATCTAGCTTG AAATCGGGTT CGGATACGGG TGGAGTTTCA     1380
Gly Val>

AATTGGTGTG TTATGGAATC CCAACTTAAT CGTGTTTAGG GGTGGGATCC AATTGTGTGA 1440

TACATTACAG AGCATGGTTG TGGATTGTTT TCTCATATGT TTTGATTGAC TTGCTTGATA 1500

CATTGGATGA TTCGATAAGG TGACCGGTTT ACCTGGGTAT CCAACCATCA TCCGATTACT 1560

TTTTAATAAT TATTTGTTTC TTCTTTATGT TGTCTGTCTT TTTGTTTCTT GATCTATAAC 1620

ATTATATTTG CCCAAATTTT CGCATTTTCC ATATGTAGCT TATATATGTA TATATATATT 1680

CAATAAAGTA TATTGATTTA GCAGATGATT TGTGTATATA TTTAAATCAA ATCAAACATT 1740

AATGATCATT CACTAGCGTC TTAATCTTGA AAAATTCATC AACGGTTATC CTTTGCAGCA 1800

TATATAAAAA AAATTGCCAA CCCTATGCTT TTACACCTAA TTCAAGGGAT AACATAAGTC 1860

GATTAAAACG A                                                      1871
```

FIGURE 7B

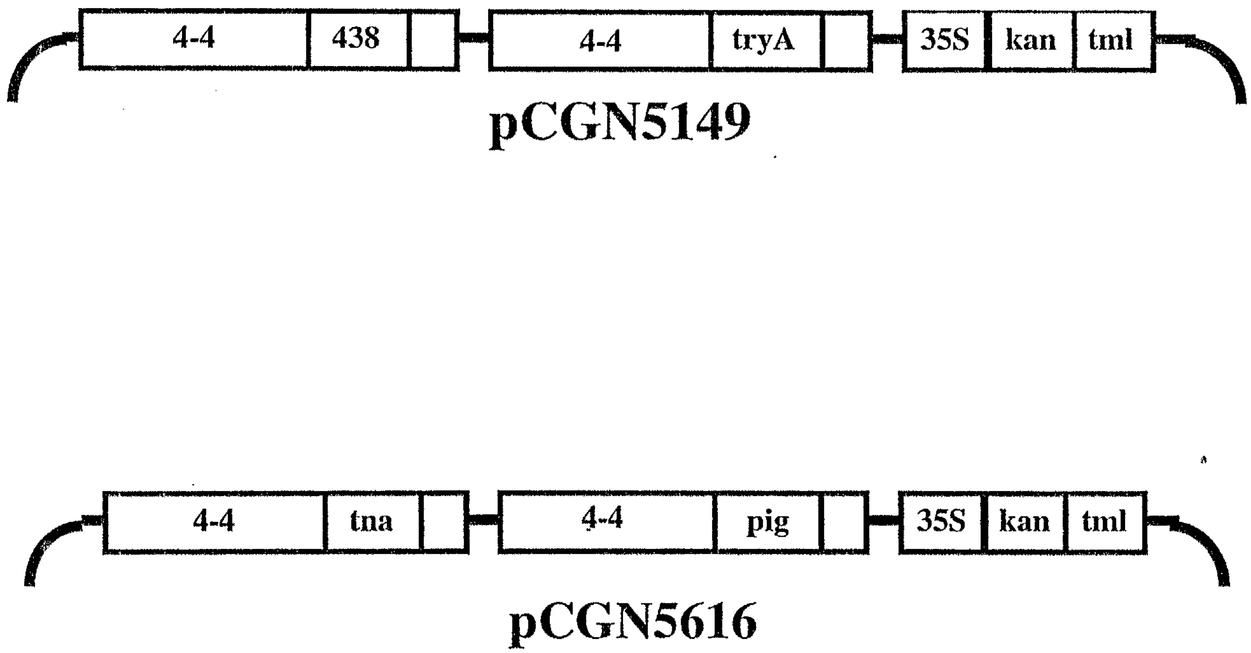

4-4
438
4-4
tryA
35S
kan
tml
pCGN5149
4-4
tna
4-4
pig
35S
kan
tml
pCGN5616

FIGURE 8

Coker 130	Yxy, Y	Yxy, x	Yxy, y	Lab, L	Lab,a	Lab,b	LCh, L	LCh,C	LCh, h
1	80.35	.3206	0.3266	91.84	0.16	5.51	91.84	5.51	88.4
2	77.62	.3232	0.3282	90.6	0.66	6.45	90.6	6.48	84.2
3	80.98	.3197	0.3257	92.12	0.13	5.04	92.12	5.04	88.6
4	80.16	.3200	0.3255	91.75	0.35	5.00	91.75	5.01	86.1
5	77.03	.3220	0.3271	90.33	0.61	5.84	90.33	5.87	84.1
6	73.67	.3258	0.3293	88.76	1.35	7.14	88.76	7.26	79.4
7	82.43	.3178	0.3237	92.76	0.15	4.05	92.76	4.05	87.9
8	82.21	.3196	0.3255	92.66	0.19	4.99	92.66	4.99	87.9
9	81.19	.3194	0.3241	92.21	0.77	4.42	92.21	4.48	80.2
10	76.11	.3243	0.329	89.9	0.74	6.89	89.9	6.92	84
11	82.28	.3178	0.3236	92.69	0.19	4.00	92.69	4.00	87.3
TOTAL	874.03	3.5302	3.5883	1005.62	5.30	59.33	1005.62	59.61	938.10
MEAN	79.46	.3209	.3262	91.42	0.48	5.39	91.42	5.42	85.28
S.D.	2.91	.0026	.0020	1.33	0.38	1.08	1.33	1.11	3.22
RANGE	82.43-73.67	.3858-.3178	0.3293-.3236	92.76-88.76	1.35-.13	7.14-4.00	92.76-88.76	7.26-4.00	88.6-79.4
AVER DEV.	2.44	.0021	.0017	1.11	0.31	0.88	1.11	0.90	2.64

Coker 130	Hunter L	Hunter a	Hunter B
1	89.63	0.15	5.42
2	88.10	0.66	6.27
3	89.98	0.13	4.98
4	89.53	0.36	4.94
5	87.76	0.61	5.69
6	85.83	1.35	6.85
7	90.79	0.15	4.03
8	90.67	0.19	4.95
9	90.10	0.78	4.38
10	87.23	0.75	6.65
11	90.70	0.19	3.98
TOTAL	980.32	5.32	58.14
MEAN	89.12	0.48	5.29
S.D.	1.65	0.39	0.99
RANGE	90.79-85.83	1.35-.13	6.85-3.98
AVER DEV.	1.37	0.31	0.81

FIGURE 9

5148	Yxy, Y	Yxy, x	Yxy, y	Lab, L	Lab,a	Lab,b	LCh, L	LCh,C	LCh, h
68-1	60.76	0.34	0.35	82.24	2.32	15.11	82.24	15.28	81.3
68-1	61.89	0.34	0.34	82.82	1.97	14.31	82.85	14.44	82.2
50-2-1	78.39	0.3324	0.3375	90.95	0.68	11.29	90.95	11.31	86.6
50-2-1									
(lint fiber)	21.49	.3155	0.3489	53.48	-8.01	7.97	53.48	11.29	135.2

5148	Hunter L	Hunter a	Hunter B
68-1	77.94	2.25	13.35
68-1	78.67	1.92	12.75
50-2-1	88.53	0.69	10.71
50-2-1			
(lint fiber)	46.35	-6.35	6.06

FIGURE 10

5149	Yxy, Y	Yxy, x	Yxy, y	Lab, L	Lab,a	Lab,b	LCh, L	LCh,C	LCh, h
68-1	65.75	0.3351	0.34	84.86	0.72	11.9	84.86	11.92	86.6
68-1	62.54	.3458	0.3474	83.19	2.14	15.84	83.19	15.98	82.4
68-1	62.56	0.3458	0.3474	83.2	2.14	15.85	83.2	15.99	82.4
8-1	84.72	.3196	0.3278	93.76	0.89	5.87	93.76	5.93	98.6
68-1	64.97	.3316	0.3354	84.46	1.17	9.81	84.46	9.87	83.3
17-2	64.42	.3423	0.3436	84.18	2.26	14.19	84.18	14.36	81
17-3	60.97	.3475	0.3475	82.36	2.74	16.03	82.36	16.26	80.4
17-15-1	64.02	.3433	0.3444	83.97	2.34	14.57	83.97	14.75	80.9
21-1	59.32	0.3443	0.3445	81.46	2.64	14.41	81.46	14.64	79.7
21-3	63.64	0.34	0.3409	83.77	2.4	12.89	83.77	13.11	79.5
21-6	67.12	0.3372	0.3394	85.56	1.88	12.15	85.56	12.29	81.3
50-3-1	61.26	0.3502	0.3511	82.51	2.4	17.63	82.51	17.79	82.3
67-1	64.34	0.3434	0.3442	84.13	2.48	14.58	84.13	14.78	80.4
68-1	64.12	0.3442	0.3447	84.02	2.58	14.85	84.02	15.07	80.2
68-2	70.21	0.3428	0.3447	87.09	2.05	15.04	87.09	15.17	82.3
68-3	63.81	0.3457	0.3468	83.86	2.35	15.76	83.86	15.93	81.6

5149	Hunter L	Hunter a	Hunter B
68-1	81.08	0.71	10.89
68-1	79.08	2.08	14
68-1	79.09	2.09	14.02
8-1	92.04	0.91	5.81
68-1	80.6	1.15	9.06
17-2	80.25	2.21	12.75
17-3	78.08	2.68	14.09
17-15-1	80.01	2.29	13.05
21-1	77.01	2.56	12.73
21-3	79.77	2.35	11.65
21-6	81.92	1.86	11.14
50-3-1	78.26	2.33	15.36
67-1	80.2	2.43	13.07
68-1	80.07	2.53	13.28
68-2	83.79	2.04	13.68
68-3	79.87	2.3	14

FIGURE 11

5616	Yxy, Y	Yxy, x	Yxy, y	Lab, L	Lab,a	Lab,b	LCh, L	LCh,C	LCh, h
11-1	72.26	0.3215	0.3254	88.09	1.1	5.06	88.09	5.17	77.8
11-2	58.69	0.3284	0.3335	81.12	0.6	8.36	81.12	8.38	85.9
11-2	52.78	0.3358	0.3335	77.74	3.55	9.22	77.74	9.87	69
11-1	72.03	0.3312	0.3338	87.98	1.72	9.52	87.98	9.67	79.8
11-1	72.34	0.3295	0.332	88.13	1.79	8.64	88.13	8.82	78.4
11-1	71.98	0.3295	0.3313	87.95	2.09	8.39	87.95	8.64	76.1
11-1	73.01	0.3256	0.3305	88.45	0.68	7.51	88.45	7.54	84.9
17-1-2	75.85	0.3274	0.3306	89.78	1.52	7.94	89.78	8.08	79.3
17-3-1	72.6	0.3271	0.3303	88.25	1.48	7.66	88.25	7.8	79.1
17-4-1	69.02	0.3352	0.3377	86.51	1.78	11.37	86.51	11.5	81.2
25-11-1	69.5	0.3364	0.3401	86.75	1.26	12.41	86.75	12.47	84.2
25-28-1	72.21	0.3324	0.3343	88.06	2.09	9.9	88.06	10.11	78.2
25-36-2	70.46	0.3327	0.3353	87.22	1.73	10.22	87.22	10.36	80.5
35-35-1	75.59	0.3268	0.3299	89.66	1.56	7.58	89.66	7.73	78.4
50-12-1	73.13	0.3284	0.3316	88.5	1.46	8.36	88.5	8.48	80.1
KS-11-2	65.33	0.3371	0.3388	84.65	2.07	11.83	84.65	12	80.1

5616	Hunter L	Hunter a	Hunter B
11-1	85	1.09	4.89
11-2	76.61	0.58	7.64
11-2	72.64	3.38	8.22
11-1	84.87	1.72	8.97
11-1	85.05	1.79	8.2
11-1	84.84	2.08	7.96
11-1	85.44	0.67	7.18
17-1-2	87.08	1.52	7.62
17-3-1	85.2	1.48	7.31
17-4-1	83.07	1.76	10.52
25-11-1	83.36	1.25	11.43
25-28-1	84.97	2.08	9.32
25-36-2	83.94	1.72	9.56
35-35-1	86.94	1.57	7.29
50-12-1	85.51	1.46	7.96
KS-11-2	80.82	2.04	10.81

FIGURE 12

BC	Yxy, Y	Yxy, x	Yxy, y	Lab, L	Lab,a	Lab,b	LCh, L	LCh,C	LCh, h
12 Green	33.34	0.3779	0.3717	66.01	4.24	24.18	66.01	24.54	80.1
22 Brown	38.18	0.3778	0.3662	68.15	6.18	23.31	68.15	24.11	75.2
3 Red	24.23	0.4055	0.3728	56.31	10.96	25.52	56.31	27.77	66.9
4 Ivory	46.84	0.3657	0.3599	74.08	4.6	21.13	74.08	21.62	77.8

BC	Hunter L	Hunter a	Hunter B
12 Green	59.44	3.79	17.92
22 Brown	61.78	5.62	17.69
3 Red	49.22	9.42	17.14
4 Ivory	68.43	4.31	17.02

FIGURE 13